*Early*CDT®-Lung

Scientific Overview of *Early*CDT-Lung Test

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*Early*CDT-Lung Overview

GeneNews has licensed *Early***CDT-Lung** from Oncimmune Ltd, a UK-based company with a US subsidiary, for marketing, sale and distribution of this test throughout the U.S. *Early***CDT-Lung** is a sophisticated blood test that, in combination with Low Dose Lung CT, aids physicians in the early detection of lung cancer in patients who are at elevated risk for the development of lung cancer.The *Early***CDT-Lung** test is a blood test based on enzyme linked immunosorbent assay (ELISA) principles. It uses microtiter plates coated with a set of serial dilutions of recombinant antigens. Samples are judged to be positive for the presence of autoantibodies when they (i) show a dose response to the antigen titration series and (ii) have a signal above either of the moderate or high cut-off points for an assay to one or more of the antigens. Test cut-off values were selected using Monte Carlo search methods and then tested prospectively on independent data sets.

During tumorigenesis, normal cells produce a number of novel, aberrantly expressed or mutated proteins (autoantigens) which are recognized by the immune system as 'non-self' and elicit the production of antibodies against them ('autoantibodies'). Autoantibodies arise in the early stages of lung cancer development, can also be present at later stages, and exist in sufficient quantity and size to be measurable in blood even when the tumor may be small and/or localized. Elevated levels of autoantibodies beyond the two predetermined cutoffs are indicative of disease, whereby the test indicates increased probability of lung cancer, necessitating a follow-up test(s) [e.g., Computed Tomography (CT)].

Elevation of any one of the seven autoantibodies (NY-ESO-1, CAGE, GBU4-5, MAGE-A4, SOX2, p53,HuD) above a predetermined cut-off value suggests that a tumor may be present or will develop.3,4,5 If the autoantibody levels all lie below the cutoff the patient is still considered high risk because of associated risk factors, such as smoking, whereby monitoring via follow- up testing with *Early***CDT-Lung** is recommended, along with adherence to current lung cancer screening recommendations by the US Preventive Services Task Force (USPSTF).

The key advantage of *Early***CDT-Lung** is its ability to detect lung cancer earlier, and with higher sensitivity and specificity than low dose CT alone, which is the current standard diagnostic imaging test.

*Early*CDT-Lung significantly aids the assessment of malignancy risk in pulmonary nodules

Lung cancer screening with computerized tomography (CT) scans is leading to a significant increase in the number of patients diagnosed with lung nodules. Lung nodules – small masses of tissue in the lung, which appear as round, white shadows on a chest X-ray or CT scan – are common. Over 95% of lung nodules on CT scans are false positives, i.e. not cancer. Of those nodules between 8mm to 20mm, about 50% are intermediate risk for cancer (10-65%).[1] A lung nodule that is 30mm or larger is more likely to be cancerous than a smaller lung nodule. If a lung nodule is detected on an imaging test, physicians might compare the current imaging scan with a previous one. If the nodule on earlier images hasn't changed in size, shape or appearance in two years, it's probably noncancerous.

*Early***CDT-Lung** significantly aids the assessment of malignancy risk in pulmonary nodules. *Early***CDT-Lung** can help reduce the number of patients in 'watchful waiting' and aid early lung cancer detection, leading to earlier intervention and better patient outcomes. In a cohort of 296 patients with a pulmonary nodule(s), a positive *Early***CDT-Lung** test was associated with a more than 2-fold increase in risk of lung cancer for nodules 4 to <20 mm (n=196)[3].

*Early***CDT-Lung** significantly improves Positive Predictive value (PPV) for the assessment of risk of lung nodule malignancy. A positive *Early***CDT-Lung** result can be used to 'rule-in' lung nodules as malignant: nearly 4 out of 5 positive results are a true cancer with a High Level result, and 1 in 1.7 for a Moderate Level result. For Intermediate (10% – 65%, 8-20mm in diameter) risk nodules, a High Level *Early***CDT-Lung** result shifts ALL nodules to Intervention risk (> 65%). A High Level *Early***CDT-Lung** result has high Specificity (98%), PPV>78% and 84% Accuracy in patients with nodules.[2,4] A Moderate Level *Early***CDT-Lung** result adds more than 25% risk of malignancy and will shift some nodules from Intermediate to Intervention risk level. A 'No Significant Level of Autoantibodies Detected' *Early***CDT- Lung** result does not change the patient's overall risk of lung cancer, for it is not a rule out test. With this result, clinicians should continue with their previously selected treatment pathway for the patient.

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*Early*CDT-Lung Validation

A key ingredient to *Early***CDT-Lung**'s success is its extensive scientific and clinical validation studies. More than 120,000 patient samples were run and 12 million data points analyzed to validate the technical and clinical performance of *Early***CDT-Lung**. Since then, over 150,000 commercial tests have also been run in the US laboratory.

Early**CDT-lung**, in the 12,000 patient National Health Service, Scotland study, has shown that it can detect lung cancer as much as 2 or more years before it may be seen via LDCT. A raised risk score, followed up aggressively for 2 years, demonstrated a sensitivity of 81%, while maintaining a specificity of 91%. This contrasts with the validation studies where, with only a six month follow up, sensitivity was 40%. The difference is in aggressive follow-up for a significant enough time to allow LDCT to see it. LDCT, while the standard for screening for lung cancer, typically will not differentiate well below a range of 2-4 mm. Also, there is an issue with differentiating between benign and malignant growths, with a false positive rate of 96%. A combination of Early**CDT**-**lung** and LDCT should deliver a superior result than LDCT alone.

In a cohort of 296 patients with a pulmonary nodule(s), a positive *Early***CDT-Lung** test was associated with a more than 2-fold increase in risk of lung cancer for nodules 4 to <20 mm (n=196).[5]
Combining *Early***CDT-Lung** with current malignancy risk assessment methods for pulmonary nodules has shown that a positive *Early***CDT-Lung** result can add to the interpretation and re- classify an intermediate risk nodule to expedite intervention.[6]

For CT-identified lung nodules, *Early***CDT-Lung**'s combined High and Moderate Level test result performance of 93% Specificity, 41% Sensitivity, PPV 59% (1 in 1.7) and 83% Accuracy, make it a valuable 'rule-in test' for lung cancer and a key tool to assess nodule malignancy risk.[7]

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7. Figures based on lung cancer prevalence 20%

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Audit of the autoantibody test, *Early*CDT®-Lung, in 1600 patients: An evaluation of its performance in routine clinical practice. Jett JR, Peek LJ, Fredericks L, Jewell W, Pingleton WW and Robertson JFR. Lung Cancer 2014;83:51-55.

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Audit of the autoantibody test, EarlyCDT®-Lung, in 1600 patients: An evaluation of its performance in routine clinical practice

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abstract

Objectives: EarlyCDT®-Lung may enhance detection of early stage lung cancer by aiding physicians in assessing high-risk patients through measurement of biological markers (i.e., autoantibodies). The test's performance characteristics in routine clinical practice were evaluated by auditing clinical outcomes of 1613 US patients deemed at high risk for lung cancer by their physician, who ordered the EarlyCDT-Lung test for their patient.

Methods: Clinical outcomes for all 1613 patients who provided HIPAA authorization are reported. Clinical data were collected from each patient's treating physician. Pathology reports when available were reviewed for diagnostic classification. Staging was assessed on histology, otherwise on imaging.

Results: Six month follow-up for the positives/negatives was 99%/93%. Sixty-one patients (4%) were identified with lung cancer, 25 of whom tested positive by EarlyCDT-Lung (sensitivity = 41%). A positive EarlyCDT-Lung test on the current panel was associated with a 5.4-fold increase in lung cancer incidence versus a negative. Importantly, 57% (8/14) of non-small cell lung cancers detected as positive (where stage was known) were stage I or II.

Conclusions: EarlyCDT-Lung has been extensively tested and validated in case–control settings and has now been shown in this audit to perform in routine clinical practice as predicted. EarlyCDT-Lung may be a complementary tool to CT for detection of early lung cancer.

1. Introduction

Lung cancer currently causes more deaths from cancer in the world than any other tumor type, and projections over the next 20 years indicate this is likely to continue unless substantial progress is made in areas such as screening, early detection, treatment and prevention. The National Lung Screening Trial (NLST) addressed the question of CT screening and early detection in a large randomized trial and reported a 20% reduction in lung cancer mortality [1]. This provided level 1 evidence and confirmation of previous non-randomized trials of CT screening [2–5] that reported more detection of early stage disease and prolonged survival.

The fact that we now know that screening and early detection saves lives from lung cancer is in many ways only the start of the process of developing a cost effective early detection program. A screening program based only upon CT as demonstrated by the NLST study has numerous problems, including a high number of benign nodules identified (i.e., false positives; e.g., 96.4% of the positive results in the NLST study were benign) [1,2,6,7], the lingering question of what to do after 3 annual screens, and the fact that only ~30% of all lung cancer patients would meet the NLST entry criteria (i.e., 55–74 years of age, 30 pack-years smoking history, and if an ex-smoker, must have quit within the last 15 years) [1]. One recent publication from a single US center focused on patients presenting with early stage lung cancers and aimed to address the question of the percentage of patients with early stage lung cancer who fulfilled the NLST criteria. Based on 267 patients with early stage disease, less than half met the NLST high risk criteria. Since the majority of these patients were not considered high-risk by the NLST criteria, they would not be covered under current screening paradigms [8].

It therefore seems that a requirement for an effective early detection program would be a biological test that would increase

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Table 1
Breakdown of age, gender and 5-year lung cancer risk [10] for the groups tested on the 6AAB and 7AAB EarlyCDT-Lung panels.

	Overall			6AAB			7AAB		
	Total number of patients	Age (median; range)	Mean 5-year lung cancer risk (number assessable[a])	Total number of patients	Age (median; range)	Mean 5-year lung cancer risk (number assessable[a])	Total number of patients	Age (median; range)	Mean 5-year lung cancer risk (number assessable[a])
Male	676 (42%)	63; 30–95	4.4% (613)	363 (48%)	63; 30–85	4.5% (332)	313 (36%)	62; 38–95	4.1% (281)
Female	937 (58%)	61; 31–92	2.2% (868)	389 (52%)	62; 31–92	2.5% (350)	548 (64%)	60; 35–89	1.9% (518)

[a] In some cases, patient demographic information was incomplete; therefore, 5-year lung cancer risk could not be calculated.

the pre-test probability of lung cancer in a high risk population – the pre-test probability being based either on demographic factors (e.g., age and smoking history), imaging findings (e.g., lung nodules) or both. A biological test that is performed on a peripheral blood sample would have clear advantages, including patient compliance, convenience and cost savings. EarlyCDT-Lung is a blood test that measures autoantibodies to lung cancer-associated antigens. It was developed to aid physicians in the early detection of lung cancer in a high-risk population. EarlyCDT-Lung was introduced clinically in a limited manner; as part of the limited release of the test a clinical audit program was established for individuals who gave consent for follow-up in accordance with the HIPAA Privacy Rule. The primary purpose of the audit was to confirm that the characteristics of the test, as reported in the training and validation case–control studies, were reproducible in routine clinical practice. This manuscript reports clinical outcomes at 6 months following EarlyCDT-Lung for the first 1600 patients whose physicians ordered the test and where the patient gave informed consent to be part of the audit program.

2. Patients and methods

2.1. Audit population

The first 1699 patients for whom US physicians ordered EarlyCDT®-Lung are described here. The tests were ordered by 810 unique physicians in 720 different practices throughout 48 US states. As this is an audit of clinical practice, we are reporting the physicians' use of the test and not a prospective study in a population defined by inclusion and exclusion factors. Of these 1699 patients, 1613 (95%) signed a HIPAA authorization permitting the ordering physician to disclose health information to Oncimmune®, and it is this group that has been followed in this audit for clinical outcomes to confirm EarlyCDT-Lung test characteristics in routine practice.

The EarlyCDT-Lung panel was modified in November 2010 from a 6 autoantibody (6AAB) panel to a panel measuring 7 autoantibodies (7AAB) to improve specificity of the test, which has been previously reported [9]. This report does not focus again on this point, but the inclusion of patients tested on both the 6AAB and 7AAB panels in this dataset does allow comparison of these two sub-groups in routine practice. The patient demographics of the overall audit population ($n = 1613$) and the 6AAB ($n = 752$) and 7AAB ($n = 861$) panel groups are shown in Table 1 along with the 5-year lung cancer risk for each group, which was calculated using a modified version of the Spitz model that takes into account demographic risk factors such as gender, age and smoking history [10].

2.2. EarlyCDT-Lung assay

EarlyCDT®-Lung is a physician-ordered blood test that serves as a tool to aid in early detection of lung cancer in high-risk patients. The test is performed only in Oncimmune's CLIA laboratory (De Soto, KS). The technology has been extensively validated and has been shown to be technically and clinically robust [9,11–13]. EarlyCDT-Lung detects the presence of AABs to a panel of lung cancer-associated antigens using a semi-automated indirect ELISA-based method. A test result was reported as positive if the antigen titration series showed a dose response and any one or more AAB levels were elevated above the clinical cut-off.

Table 2
Six-month follow-up percentages for patients testing positive and negative by EarlyCDT-Lung.

	Positive follow-up %	Negative follow-up %
Overall	99	93
6AAB	100	97
7AAB	98	91

Testing of all patient specimens by EarlyCDT-Lung was performed in Oncimmune's CLIA laboratory, including the data handling and calculation of the test result, which was performed by the Oncimmune laboratory information management system (LIMS); final test results were generated and reported to individual physicians. All EarlyCDT-Lung tests were performed prospectively upon receiving the physician's order, and the results were reported back to the physician without knowledge of the patient's clinical outcome, which was subsequently obtained as part of this audit.

2.3. Audit plan

Demographic data were requested as part of the EarlyCDT-Lung test requisition form. These data were considered in the audit. Additionally, clinical follow-up data on patients who provided HIPAA authorization were collected from their treating physician. In patients with a positive EarlyCDT-Lung test, contact was made with physicians immediately following the reporting of the EarlyCDT-Lung result and maintained until the physician indicated that a diagnosis had been reached or a follow-up plan decided (i.e., anticipated timing of imaging, biopsy, surgery, etc.); this was usually within 2–3 months of the EarlyCDT-Lung test. Subsequent follow-up on patients with a positive EarlyCDT-Lung test was then structured around the physician-described follow-up plan. Information concerning whether a patient was diagnosed with cancer was requested from physicians for all individuals regardless of test result at 6 months after the test. This timeframe was chosen (i) because it was felt to represent a timeframe within which the immediate value of a positive test result could be assessed, (ii) it allowed time for all patients with a negative EarlyCDT-Lung test to present with lung cancer in order to reduce the chance of observer bias in preferentially following up individuals with a positive EarlyCDT-Lung test result. One patient with a positive test was diagnosed just outside the 6 month period: this patient has been included since they were being actively investigated during the six month period for a lesion identified on imaging as being suspicious of lung cancer. The overall percentage of individuals followed-up at six months in the positive and negative EarlyCDT-Lung groups was 99% and 93%, respectively (Table 2); these data are also further broken down by the 6AAB and 7AAB groups (Table 2).

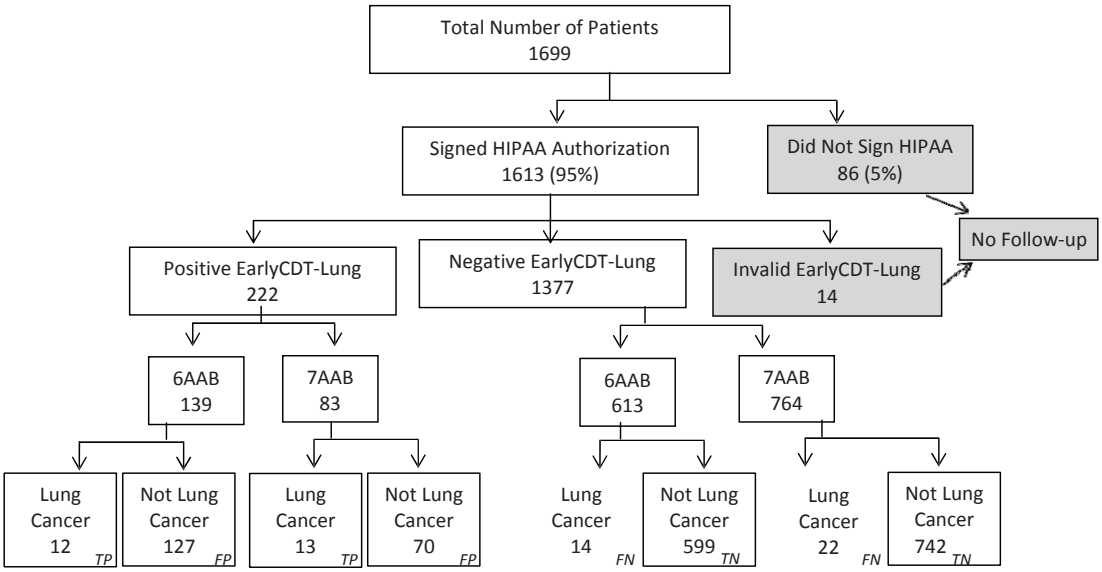

Fig. 1. Breakdown of patients considered in this audit, by EarlyCDT-Lung result, test panel and clinical outcome. [6AAB: 6 autoantibody EarlyCDT-Lung panel; 7AAB: 7 autoantibody EarlyCDT-Lung panel; TP: true positives; FP: false positives; TN: true negatives; FN: false negatives.]

This report, therefore, focuses on the initial presentation and outcomes of all patients within 6 months following testing by EarlyCDT-Lung. Wherever possible, histology/cytology reports were reviewed and considered for diagnostic classification; some patients did not have a tissue diagnosis but were diagnosed, for example, based on imaging reports. It was decided from the start of the audit that if a physician diagnosed a lung cancer, then only in circumstances where there was specific proof to the contrary, and this was confirmed by an external expert, would the diagnosis by the treating physician not be accepted; this rule was applied for all patients regardless of EarlyCDT-Lung result.

2.4. Statistical analyses

The EarlyCDT-Lung test performance is presented in terms of standard test characteristics, such as sensitivity (the percentage of true positives) and specificity (the percentage of true negatives). Positive predictive value (PPV; the probability of cancer given a positive test result) was also calculated. These analyses were performed using Microsoft Excel. Comparison of sensitivity and specificity of EarlyCDT-Lung for the 6AAB and 7AAB groups is also presented; these comparisons were made using chi-squared tests.

3. Results

Of the 1613 test results, there were 14 patients where the test result was declared 'Invalid' (by pre-determined criteria, as outlined in the laboratory's standard operating procedures) on the report sent to the treating physician. There were 222 patients who tested positive (14%) and 1377 tested negative (86%) (Fig. 1). The percent positive for the 6AAB and 7AAB panels was 18% (n = 139) and 10% (n = 83), respectively.

Sixty-one patients (4%) were diagnosed with lung cancer within 6 months following EarlyCDT-Lung, 25 of whom tested positive by EarlyCDT-Lung (i.e., 25 true positives and 36 false negatives; sensitivity = 41%). There were 1341/1538 patients not diagnosed with lung cancer who tested negative (i.e., 1341 true negatives and 197 false positives; specificity = 87%). The correlation between the EarlyCDT-Lung result and clinical outcome in terms of diagnosis within six months after having taken the EarlyCDT-Lung test is shown in Fig. 1 and Table 3. Comparing performance of the two panels, the 7AAB panel showed highly statistically significant (p < 0.0001) improvements in specificity over the 6AAB panel with 91% specificity for the 7AAB panel (i.e., 742 true negatives and 70 false positives) and 83% specificity for the 6AAB panel (i.e., 599 true negatives and 127 false positives). The sensitivities of the 6AAB and 7AAB panels were not statistically different (p = 0.5): 46% (i.e., 12 true positives and 14 false negatives) versus 37% (i.e., 13 true positives and 22 false negatives), respectively. The improvement in PPV offered by the 7AAB panel was nearly 2 better than the previous 6AAB panel: 16% (1 in 6.4) for the 7AAB panel versus 9% (1 in 11.6) for the 6AAB panel (Table 3).

Table 3
Clinical performance of the 6AAB and 7AAB panels, calculated from the clinical audit dataset with 6 month follow-up for all patients.

	Specificity (%; 95% CI)[a]	Sensitivity (%; 95% CI)[b]	PPV
Overall	1341/1538 (87%; 85–89%)	25/61 (41%; 29–54%)	1 in 8.9 (11%)
6AAB	599/726 (83%; 79–85%)	12/26 (46%; 27–67%)	1 in 11.6 (9%)
7AAB	742/812 (91%; 89–93%)	13/35 (37%; 21–55%)	1 in 6.4 (16%)

95% CI: 95% confidence interval, calculated in SAS using the Clopper-Pearson exact method.

[a] The 7AAB panel shows a highly statistically significant improvement in specificity of EarlyCDT-Lung (p < 0.0001).

[b] The sensitivities of the 6AAB and 7AAB panels were not statistically different (p = 0.5).

Of the 61 lung cancer cases diagnosed, 46 (75%) were non-small cell lung cancer (NSCLC), 4 (7%) were small cell lung cancer (SCLC), 1 (2%) was mixed NSCLC-SCLC, and type was unknown for 10 (16%) cases (Table 4). Of the 46 NSCLCs with a histologic diagnosis, 26 (57%) were early-stage (stage I or II), 16 (35%) were late-stage (stage III or IV) and 4 (9%) were stage unknown (Table 4). Importantly, 57% (8/14) of NSCLCs detected as positive by EarlyCDT-Lung (where stage was known) were early-stage. Stage was unknown for an additional 2 NSCLCs detected by EarlyCDT-Lung. Thirty-two NSCLCs were adenocarcinoma and 14 were squamous cell carcinoma. Only four cases of small cell lung cancer were diagnosed, which is too few to allow for further evaluation. Of the 10 patients with unknown type of lung cancer (Table 4), 9 were diagnosed clinically due to the patient's condition being too fragile for biopsy

Table 4
Breakdown of cancer type/sub-type and stage of disease for the 61 lung cancer cases.

Lung cancer type/sub-type	Number	Stage				
		I	II	III	IV	N/A
NSCLC						
Adenocarcinoma	32 (52%)	13 (41%)	1 (3%)	8 (25%)	7 (22%)	3 (9%)
Squamous	14 (23%)	8 (57%)	4 (29%)	1 (7%)	–	1 (7%)
Total (NSCLC)	46	21 (46%)	5 (11%)	9 (20%)	7 (15%)	4 (9%)
SCLC						
Small cell lung cancer (SCLC)	4 (7%)	–	–	3 (75%)	1 (25%)	–
Other						
Mixed SCLC + NSCLC	1 (2%)	–	–	1 (100%)	–	–
Unknown type[a]	10 (16%)	3 (30%)	2 (20%)	–	1 (10%)	4 (40%)
Overall total	61	24 (39%)	7 (11%)	13 (21%)	9 (15%)	8 (13%)

[a] "Unknown Type" refers to those patients with a clinical diagnosis of lung cancer who were too fragile for biopsy, had an inconclusive biopsy, declined further testing or whose records were unavailable.

(n = 4), an inconclusive biopsy (n = 3) or the patient refused diagnostic procedures (n = 2), and in 1 case the information was not accessible due to the patient's records being in storage.

4. Discussion

The performance characteristics of the EarlyCDT-Lung test in clinical practice, as demonstrated by this prospective audit, mirrors that of the extensive case–control training and validation studies previously reported [9,12–14]. This audit has confirmed that EarlyCDT-Lung detects all types of lung cancer, all stages of the disease, and performs in clinical practice with the same sensitivity and specificity measured in the case–control studies. This is, therefore, the first autoantibody test that detects early stage lung cancer as shown with prospective validation data on a large number of individuals from a routine clinical practice setting.

Furthermore, the previously reported change that was made to the panel in November 2010 (6AAB panel to 7AAB panel) [9] has proven in routine clinical practice to have reduced the number of false positives (i.e., increased specificity), while maintaining the same ability to detect lung cancers (i.e., sensitivity). This resulted in an increased PPV of EarlyCDT-Lung in routine clinical practice from 9% (1 in 11.6) with the 6AAB panel to 16% (1 in 6.4) with the 7AAB panel (Table 3). For patients with a negative EarlyCDT-Lung result on the current 7AAB panel, 22/764 (3%) were found to have a lung cancer (i.e., 1 in 34.7). Thus, a positive result on the current 7AAB EarlyCDT-Lung test panel represents, on average, a 5.4-fold increased incidence of lung cancer within 6 months.

According to the National Cancer Institute's SEER statistics, 39% of lung cancers are adenocarcinoma, 21% are squamous cell, and 14% are SCLC [15]. With the exception of a slightly higher proportion of adenocarcinoma (52%) and lower proportion of SCLC (7%) in our group, our audit findings are in line with the SEER statistics' breakdown by histological sub-type, confirming that the cohort presented here is representative of a high-risk (for lung cancer) population and is not heavily biased toward any particular type of lung cancer. These audit data also confirm the case–control validation results that EarlyCDT-Lung detects all sub-types of lung cancer.

EarlyCDT-Lung has been shown in case–control studies and now in this clinical audit to also detect early-stage lung cancer. In the group evaluated for this audit where stage was known, 57% (8/14) of NSCLCs detected by EarlyCDT-Lung were early-stage.

The results presented on the overall performance characteristics of the test (e.g., specificity and sensitivity) confirm that in routine clinical practice EarlyCDT-Lung performs as predicted from our previously reported large case–control studies. The audit results have highlighted the value of the test to physicians as an aid to detection of early lung cancer.

Until recently, there were no significant biological markers related to the individual or the lung cancer that could be measured as a blood test and used in clinical practice. EarlyCDT-Lung measures AABs to lung cancer-associated antigens; it is biologically based and has been reported to be independent of a patient's demographics and smoking history [16]. Its high specificity and PPV make it a potentially complementary tool for use in conjunction with CT to evaluate a patient at high risk for lung cancer. For example, if a pulmonary nodule is identified on a CT scan and the EarlyCDT-Lung test is positive, the probability of malignancy is significantly increased (manuscript in preparation). In addition, if a patient who falls just outside the NLST criteria for CT screening tests positive by EarlyCDT-Lung, then their risk of lung cancer would be increased to a level that would now make them appropriate for CT screening. However, it is important to note that due to the relatively low sensitivity (~41%) of EarlyCDT-Lung, a negative test result does not rule out lung cancer in either scenario; in the case of the pulmonary nodule and a negative EarlyCDT-Lung result, the physician would continue to follow the current recommendations for follow-up CT scanning per the Fleischner Guidelines [17], and in the second scenario with a negative EarlyCDT-Lung result, the physician would continue monitoring the patient's health according to standard procedures, as they would have done in the absence of the EarlyCDT-Lung test.

Two prospective clinical trials are currently on-going – one in the US (assessing the value of the test in conjunction with CT) and a second in the UK (assessing the value of the test as a pre-CT screening tool).

5. Summary

This is the first biologically based blood test for lung cancer detection that has been extensively tested and validated in case–control settings and has now been shown to perform as predicted in clinical practice. The population on whom the test was used was high risk with 4% diagnosed with lung cancer within 6 months following EarlyCDT-Lung. A positive result on the current 7AAB EarlyCDT-Lung test was associated with a 5.4-fold increase in incidence of lung cancer compared to a negative test.

Conflict of interest statement

J.R. Jett has a research grant from Oncimmune. L.J. Peek is an employee of Oncimmune USA LLC. L. Fredericks, W. Jewell and W.W. Pingleton are consultants to Oncimmune USA LLC. J.F.R.

Robertson is Chief Scientific Officer and a shareholder of Oncimmune Ltd., a University of Nottingham spinout company.

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RESEARCH ARTICLE

*Early*CDT®-Lung test: improved clinical utility through additional autoantibody assays

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Abstract Tumor-associated autoantibodies (AAbs) have been described in patients with lung cancer, and the *Early*CDT®-Lung test that measures such AAbs is available as an aid for the early detection of lung cancer in high-risk populations. Improvements in specificity would improve its cost-effectiveness, as well as reduce anxiety associated with false positive tests. Samples from 235 patients with newly diagnosed lung cancer and matched controls were measured for the presence of AAbs to a panel of six (p53, NY-ESO-1, CAGE, GBU4-5, Annexin I, and SOX2) or seven (p53, NY-ESO-1, CAGE, GBU4-5, SOX2, HuD, and MAGE A4) antigens. Data were assessed in relation to cancer type and stage. The sensitivity and specificity of these two panels were also compared in two prospective consecutive series of 776 and 836 individuals at an increased risk of developing lung cancer. The six-AAb panel gave a sensitivity of 39% with a specificity of 89%, while the seven-AAb panel gave a sensitivity of 41% with a specificity of 91% which, once adjusted for occult cancers in the population, resulted in a specificity of 93%. Analysis of these AAb assays in the at-risk population confirmed that the seven-AAb panel resulted in a significant increase in the specificity of the test from 82 to 90%, with no significant change in sensitivity. The change from a six- to a seven-AAb assay can improve the specificity of the test and would result in a PPV of 1 in 8 and an overall accuracy of 92%.

Keywords Autoantibodies · Lung cancer · Lung cancer diagnosis

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Introduction

Patients with lung cancer, both non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC), can mount a humoral immune response to their cancer [1–5]. Autoantibodies (AAbs) have been described not only at the time of initial diagnosis of lung cancer [1, 2], but also, in some cases, up to 5 years before the cancer is diagnosed [6–8]. There is now level 1 evidence from the US National Lung Screening Trial that earlier diagnosis saves lives; this randomized control trial reported a 20% reduction in lung cancer mortality, following CT screening of high-risk individuals [9].

An AAb assay for lung cancer (*Early*CDT®-Lung), which was technically and clinically validated using three separate case–control study populations, has re- cently been reported [1, 10]. In these publications, AAbs to six tumor-associated antigens (p53, NY-ESO- 1, Annexin I, CAGE, GBU4-5, and SOX2) were mea- sured and identified up to 40 % of all lung cancers in the disease groups, with a specificity of 90 % (non- cancer controls individually matched to lung cancer sera by age, gender, and smoking history) [1, 10]. Further confirmation of the sensitivity and specificity of the test for lung cancer using four new, independent sample sets (*n*0574 newly diagnosed lung cancers plus controls) was recently reported [11], with no significant difference in positivity for *Early*CDT-Lung among different cancer stages being seen. The performance of the test (in terms of precision and analytical linearity [10]) is such that it is now commercially available to clinicians, to assist them in the early detection of lung cancer in combination with imaging techniques.

We report here the analysis of two additional and well-described cancer-associated antigens, MAGE A4 and HuD (n-ELAV), which are known to have particular associations with lung cancer. The MAGE gene family belongs to the chromosome X-clustered cancer/testis antigens, and the members of the MAGE A family encode proteins with 50 to 80% sequence identity to each other. The overexpression of these MAGE antigens has been described in a number of cancers including lung cancer [12, 13], and MAGE A4 has been proposed as a potential therapeutic target for immunotherapy [14]. The diagnostic potential of MAGE A4 AAb measurement has not been reported previously. HuD is a member of a family of onconeuronal RNA-binding proteins known for stabilizing RNA. It is normally expressed only on terminally differentiated neurons where it is involved in the development and maintenance of the nervous system [15–17]. Anti-Hu antibodies are often found associ- ated with paraneoplastic encephalomyelitis or sensory neuropathy, and these antibodies have been described in neuroendocrine tumors of the lung, in particular SCLC [18–20]. In fact, 17% of patients with SCLC have been described as having elevated levels of AAbs to HuD when compared to matched controls [20].

This manuscript reports an improved *Early*CDT-Lung panel with the addition of these two new AAb assays (i.e., MAGE A4 and HuD), and the removal of Annexin I, first in an optimization set comprised of patients with newly diagnosed lung cancer (before any treatment) and matched controls, and secondly in a prospective sample set confirming the additive value this modification brings to the original *Early*CDT-Lung panel in the clinical setting.

Methods

Blood samples and patient details

Optimization set

Serum samples from 235 patients with lung cancer (from the UK, USA, Ukraine, and Russia), obtained at or just after histopathological confirmation of the tumor, were assayed. These 235 samples represented 87% of the lung cancers in a previously published dataset (group 3, *n* 0269) [1], which were chosen on the basis of sufficient residual sample volume being available for analysis. The lung cancers consisted of 178 NSCLCs (75.7%), 53 SCLCs (22.6 %), and 4 others (1 sarcoma, 2× bronchogenic carcinomas, and 1 undefined lung cancer). The controls were also part of the previously published sample set and consisted of 266 healthy volunteers, 235 of which were matched to the lung cancer patients for age, gender, and smoking status. This group of controls had no evidence of any current or prior cancer including non-melanoma skin cancer. All serum samples were collected following informed consent and stored at −20 or −70°C prior to analysis. This dataset was used to re-optimize the panel performance in terms of specificity following the addition of the new antigens and the removal of Annexin I.

Clinical population set

The performance of the AAb test in an independent, clinically relevant sample set is reported here using the clinical samples sent for commercial *Early*CDT-Lung measurement [1, 10]. These consisted of 776 serum samples assessed by the original six-antigen AAb assay panel (May 2009–November 2010) and a further separate but consecutive 836 serum samples assessed by the updated seven-antigen AAb assay panel (November 2010–August 2011). All samples were from individuals in North America deemed by their clinician to be at an increased risk of developing lung cancer due to age and smoking history or other factors. All sera were taken under informed consent, and all patients had signed a HIPAA release, authorizing access to their medical records.

Antigen production

Recombinant proteins were cloned into pET expression vectors (Invitrogen) and transformed into *Escherichia coli* BL21 (DE3) bacteria. The proteins p53, NY-ESO-1, CAGE, Annexin I, MAGE A4, HuD, SOX2-B, and GBU4-5 were cloned into pET21b and produced with a His tag and BirA tag [1, 10], whereas SOX2-N was cloned into pET44b and produced with a His tag and NusA tag. Negative control proteins were also produced (BirA and NusA tags alone).

The recombinant proteins were expressed in BL21 (DE3) bacteria (Novagen) and grown in terrific broth (TB), auto-induction TB media (Novagen), ECPM media, or Power Broth (Molecular Dimensions). Recombinant proteins were purified by metal chelate affinity chromatography and refolded by dialysis [10, 21]. All recombinant proteins were produced by external suppliers. Quality control tests for acceptance of protein included SDS–PAGE, Western blotting with appropriate antibodies, and analytical size exclusion chromatography.

Autoantibody detection

AAbs to the tumor-associated antigens were measured using *Early*CDT-Lung (Oncimmune USA LLC, De Soto, KS), a commercially available blood test based on ELISA principles that uses microtiter plates coated with a set of serial dilutions of recombinant antigens as previously described [10]. All assays were run blinded to the demographic data. AAbs were measured as optical density units and then expressed in calibrated reference units (RU). Positive seroreactivity for the assay was defined as (a) having evidence of a dose response to the antigen titration series and (b) an assay result above a cutoff level (described below).

Statistics

Assay data handling (calibration of OD signal to RU) was performed by the Oncimmune LLC LIMS system. Clinical performance was expressed in terms of sensitivity (the percentage of true positives) and specificity (the percentage of true negatives). Concordance (the percentage of samples with the same test outcome in two assays being compared), accuracy (the percentage of samples correctly diagnosed), and positive predictive value (PPV; the probability of cancer given a positive test result) were also calculated. This analysis was performed using Microsoft Excel. For comparison of sensitivity and specificity values, chi-squared tests were used. Forest plots of the sensitivity at fixed specificity for subgroups were prepared using 95% binomial confidence intervals. Similarly, for individual antigens, 95% binomial confidence intervals were calculated for percentage positivity (sensitivity). This analysis was performed using SPSS®.

Assessment of lung cancer risk

Underlying risk was calculated from the Spitz et al. [22] individual lung cancer risk assessment model, which captures some of the complex interactions between exposures and host susceptibility factors. The model was adapted to predict 5-year absolute risk of lung cancer, based on gender, age, and smoking history. An in-house program was used for the calculations [23].

Optimization of assay cutoffs

A fixed target specificity of 90% was selected for the panel of six AAb assays, and the cutoffs were obtained by optimizing sensitivity using a Monte Carlo direct search method [24] and validated as previously described [1, 10]. The method searches a random selection (n 010,000) of the possible sets of cutoffs and chooses the set with the highest sensitivity for the fixed specificity. For the new panel of seven assays (including the two new antigens and SOX2-B and the removal of Annexin I), a similar Monte Carlo approach was used but this time optimizing specificity for a fixed sensitivity of approximately 40%. The optimization was performed using R software.

Adjustment for lung cancers in the control populations

In order to set accurate and meaningful cutoffs for lung cancer detection tests, the results obtained from a group of individuals known to have the disease must be compared with those obtained from a group of individuals with demographic and risk factors matched to the cancer group and known to be disease free. However, obtaining a truly disease-free control group is extremely problematic since CT screening studies have shown that in any high-risk group there is a small proportion of individuals harboring undiagnosed asymptomatic lung cancer [9]. The proportion of such individuals may be as high as 2.7% in a prevalence round and 2.3% in an incidence round (referenced in [1]). For this reason, a modified lung cancer prediction model [22] was employed that allowed for the presence of occult cancers in the control population by taking into account the most important predictors for disease such as smoking status and history as well as age. The adjustment was carried out in the Monte Carlo optimization routine as described previously [1, 24] to provide accurate sensitivity and specificity values for the *Early*CDT-Lung test.

Results

Optimization set

The sensitivity and specificity of the AAb assays for 235 lung cancers are shown in Table 1 where the data are also characterized by tumor type (i.e., NSCLC and SCLC), and a summary of the demographics of the population is shown in Table 2.

Elevated AAb levels to at least one of the original six antigens in the *Early*CDT-Lung test (p53, CAGE, NY-ESO-1, GBU4-5, Annexin I, and SOX2-N), using the original published cutoffs, gave an overall sensitivity for lung cancer detection of 39% with an unadjusted specificity of 89%, while

Table 1 Sensitivity and specificity of AAb assays for the optimization set

	n	Annexin I	p53	CAGE	NY-ESO-1	GBU4-5	MAGE A4	SOX2-B	SOX2-N	HuD	Panel of 7
All LCa	235	0 (0–2)	13 (9–18)	9 (5–13)	10 (7–15)	3 (1–7)	12 (8–17)	4 (2–8)	4 (2–8)	5 (2–8)	41 (35–48)
NSCLC	178	1 (0–3)	12 (7–17)	7 (4–11)	10 (6–16)	2 (0–5)	14 (9–20)	1 (0–3)	1 (0–3)	2 (0–5)	38 (31–46)
SCLC	53	0 (0–7)	17 (8–30)	13 (5–25)	11 (4–23)	8 (2–18)	8 (2–18)	17 (8–30)	15 (7–28)	15 (7–28)	55 (40–68)
Normals	266	0 (0–2)	3 (2–6)	1 (0–3)	2 (1–5)	2 (1–4)	4 (2–7)	1 (0–3)	1 (0–3)	1 (0–3)	9 (6–13)
Specificity	266	>99	97	99	98	98	96	99	99	99	91

Data are shown as percentage positivity following the application of the adjusted cutoffs. Numbers in parentheses are the 95% confidence interval. Specificity for lung cancer detection in the normal population is also shown. Specificity is unadjusted for the presence of cancers in the control population

Panel of 7 represents AAb positivity to any one of the antigens in the new seven-AAb *Early*CDT-Lung panel: p53, CAGE, NY-ESO-1, GBU4-5, MAGE A4, SOX2-B, and HuD

elevated levels of AAbs to at least one of eight antigens tested (p53, CAGE, NY-ESO-1, GBU4-5, HuD, Annexin I, MAGE A4, and either SOX2-N or SOX2-B) with the new and optimized cutoffs gave an overall sensitivity for lung cancer detection of 42% with an unadjusted specificity of 91%. The sensitivity and specificity of the two SOX2 proteins (with a BirA or NusA tag) were assessed independently and also as part of the panel in the dataset. Concordance between the two SOX2 antigens was 99.6% with no change in the results, suggesting either of the SOX2 proteins could be substituted in the assay.

Using these optimized cutoffs, it was clear that Annexin I was no longer additive to the panel and a smaller panel of seven AAb assays (*Early*CDT-Lung (seven): p53, NY-ESO-1, CAGE, GBU4-5, MAGE A4, HuD, and SOX2-B) achieved almost identical sensitivities and specificities using these new optimized cutoffs (Table 1). This sensitivity was not dependent on the stage or grade of the cancer and was maintained at approximately 40% even in the early-stage lung cancer samples (Fig. 1). The positivity rate for individual AAb assays in the new seven-AAb panel ranged in NSCLC from 1 (for SOX2) to 14% (for MAGE A4) and in SCLC from 8 (for MAGE A4 and GBU4-5) to 17% (for SOX2 and p53), with specificity for each antigen being ≥96% (Table 1). The detection of AAbs to some antigens was, however, more specific for the detection of certain cancer subtypes; for example, MAGE A4 predicted the presence of NSCLC more often than SCLC, while the reverse was true for HuD and SOX2 (Table 1).

Table 2 Demographics of the optimization data set

Demographic data	Cancer sera	Normal sera
Number	235	266
Male/female	73%/27%	70%/30%
Age mean (median)	64.8 (65)	64.5 (65)
Current smoker/ex smoker	46%/29%	35%/54%
Nonsmoker/unknown	10/15%	11%/0%

Both cancer and normal sera were analyzed using both the six- and seven-AAb panel of assays. Normal sera were matched as closely as possible from the available samples to the cancer sera for age, gender, and smoking history

Allowing for the presence of potentially undiagnosed cancers in the high-risk control population (as described above), the seven-AAb test demonstrated an adjusted specificity and sensitivity of 93 and 41 %, respectively. This would mean that in a high-risk population (e.g., lung cancer prevalence of 2.4 % [25]), such a change in the panel would result in an improvement in the PPV of the test from 9 (1 in 11) to 13 % (1 in 8) and therefore an increase in the accuracy of the test from 89 to 92 %. For comparison, if a lower prevalence of lung cancer is assumed (e.g., 1.3% [26]), the PPV of the new *Early*CDT-Lung (seven-assay test) test would be 7% (1 in 14) with an accuracy of 92%.

Clinical population set

The performance of the assay was evaluated in a prospective series of individuals at increased risk of developing lung cancer, by auditing the clinical follow-up data alongside the *Early*CDT-Lung results for 1,612 clinical samples, run sequentially either on the original panel of six-AAb assays (776 samples) or the new panel of seven-AAb assays (836 samples). The two sets of commercial samples could not be analyzed by both the original and new panels so direct comparison of sensitivity and specificity could not be performed. The demographics of the two groups were similar in terms of mean age and range; however, the proportion of men was higher in the six-AAb assay group, as was the average risk for development of a lung cancer (Table 3).

Overall, 2.7% of these individuals (44/1,612) were diagnosed with lung cancer after having the *Early*CDT-Lung test. When the lung cancer diagnosis was analyzed according to whether the individuals were tested using the six- or seven-AAb test, 3.2% of those who were tested using the six-AAb test and 2.3% of those who were tested using the seven-

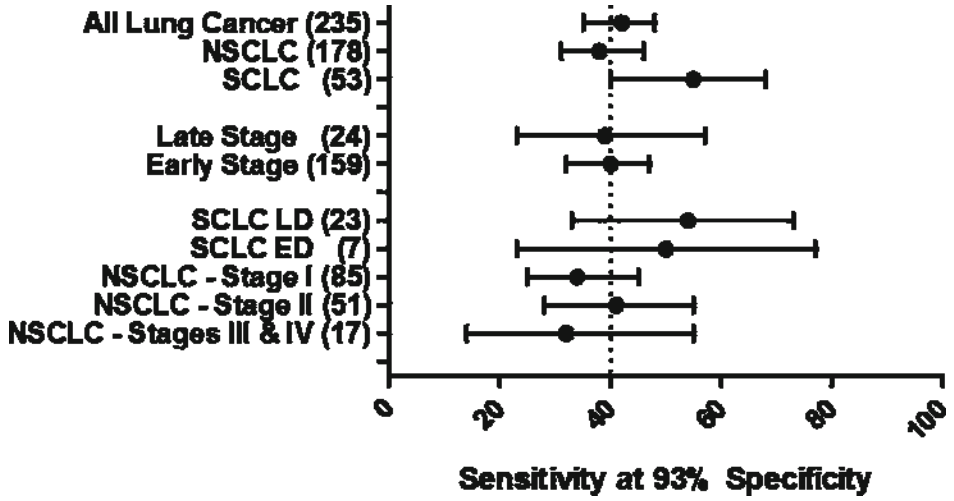

Fig. 1 Forest plot showing the sensitivity of the *Early*CDT-Lung assay at a fixed specificity of 93 % (with confidence intervals) by tumor characteristics and lung cancer stage. Positivity is defined as having an elevated AAb assay signal to any one of the antigens in the new seven-AAb *Early*CDT-Lung panel: p53, CAGE, NY-ESO-1, GBU4-5, MAGE A4, SOX2-B, and HuD. *Vertical dashed line* represents sensitivity at 40 % (all stages of lung cancer). *NSCLC* non-small cell lung cancer, *SCLC* small cell lung cancer, *LD* limited disease, *ED* extensive disease, *early stage* stage I and II NSCLCs and LD SCLCs, *late stage* stage III and IV NSCLCs and ED SCLCs. The number of samples in each group is represented in *parentheses*

AAb test had developed lung cancer, reflecting the increased risk calculated for the earlier group (Tables 3).

Of the 44 individuals diagnosed with lung cancer, 19 had elevated levels of AAbs, and the panel identified SCLC

Table 3 Demographics of the population data sets

Demographic	Number[a]	Data
Gender		Percentage
6 AAb	776	Male 48%, female 52%
7 AAb	836	Male 36%, female 64%
Total	1,612	Male 42%, female 58%
Age		Mean ([(5%ile)–median–(95%ile)]
6 AAb	776	61 [(45)–62–(77)]
7 AAb	836	60 [(43)–59–(79)]
Total	1,612	61 [(44)–61–(78)]
Ethnicity		Percentage
6 AAb	721	Caucasian 92.0%, Afr-Amer 5.7%, Hispanic 1.7%, Others 0.6%
7 AAb	811	Caucasian 90.6%, Afr-Amer 5.2%, Hispanic 2.6%, Others 1.6%
Total	1,532	Caucasian 91.3%, Afr-Amer 5.4%, Hispanic 2.2%, Others 1.1%
Smoking		Percentage
6 AAb	770	Current 47.0%, ex smoker 48.3%, nonsmoker 4.7%
7 AAb	836	Current 43.4%, ex smoker 44.3%, nonsmoker 12.3%
Total	1,606	Current 45.1%, ex smoker 46.2%, nonsmoker 8.7%
Lung cancer risk[b]		Mean [min–(5%ile)–median–(95%ile)–max]
6 AAb	770	3.1 [0.0–(0.0)–2.7–(8.3)–11.9]
7 AAb	836	2.4 [0.0–(0.0)–1.6–(7.3)–11.9]
Total	1,606	2.7 [0.0–(0.0)–2.1–(8.0)–11.9]

Demographics shown for the samples run on the 6-AAb panel, 7-AAb panel, and total (where known)

AAb autoantibody, *Afr-Amer* African-American

[a]Number denotes numbers for which data were available

[b]Lung cancer risk was calculated according to a modified Spitz et al. lung cancer prediction model [22] based on gender, age, and smoking history

Table 4 Audit of *Early*CDT-Lung test

	Number of participants	Confirmed lung cancers[a], *N* (%)	No lung cancer diagnosis[b], *N* (%)
Panel of 6-AAb assays			
Total	776	25 (3.2)	751 (96.8)
Positive AAb assay result	145	10 (6.9)	135 (93.1)
Negative AAb assay result	631	15 (2.4)	616 (97.6)
Overall panel sensitivity or specificity		Sensitivity 40%	Specificity 82%
Panel of 7-AAb assays			
Total	836	19 (2.3)	817 (97.7)
Positive AAb assay result	87	9 (10.3)	78 (89.7)
Negative AAb assay result	749	10 (1.3)	739 (98.7)
Overall panel sensitivity or specificity		Sensitivity 47%	Specificity 90%

Original six-AAb assay panel (performed on 776 samples) and new seven-AAb assay panel (performed on 836 samples) showing the number of samples that were identified as being positive or negative in the *Early*CDT-Lung test and the number of confirmed cases of lung cancer. Panel of 6 represents AAb positivity to any one of the original six-AAb *Early*CDT-Lung panel: p53, CAGE, NY-ESO-1, GBU4-5, Annexin I, and SOX2-N. Panel of 7 represents AAb positivity to any one of the new seven-AAb *Early*CDT-Lung panel: p53, CAGE, NY-ESO-1, GBU4-5, MAGE A4, SOX2-B, and HuD

[a] Number of lung cancers detected—correct as of August 2011 following CT and biopsy

[b] Number of individuals assessed as being free from lung cancer, as of August 2011

(1/2) and NSCLC (18/42), as well as both asymptomatic early-stage (stage IA and IB) and later-stage disease. Using the original panel of six-AAb assays and original cutoffs generated in our previous publication [10], the specificity/sensitivity in the first set of 776 samples was 82%/40%. Using the new panel of seven-AAb assays and the cutoffs established in the optimization set, the specificity/sensitivity in the second set of 836 samples was 90%/47% (Table 4). This change from the original six-AAb to the new seven-AAb panel represented a significant improvement in the specificity of the test for cancer detection ($p< 0.0001$) with no significant difference between the sensitivity of the two panels ($p00.63$; probably due to small numbers). Assuming the calculated risk of developing lung cancer for each group was 3.1 and 2.4%, respectively (Table 3), this would confer an increase in the PPV of the test from 1 in 15 to 1 in 10.

Discussion

Previous publications using validated, calibrated assays have confirmed the utility of measuring AAbs to tumor-associated antigens as an aid for the identification of early-stage lung cancers [1, 11]. The data presented in this manuscript reveal that improvements of such a test can be achieved by adding two new antigens and dropping one (now redundant) antigen from the *Early*CDT-Lung panel, and re-optimizing the cutoffs. This change essentially maintained the previously reported 40% sensitivity of the test for lung cancer [1] even for early-stage more treatable disease. Importantly however, it improved the specificity of the test (once adjusted for occult cancers in the population) from 90% as previously reported [1] to 93% in the same retrospective case–control (optimization) set. In a clinical setting, such an improvement would result in an increase in the PPV of the test and a 30% reduction in "false" positive tests, important benefits to both patients and clinicians.

Since the two additional antigens were added to ultimately increase the specificity of *Early*CDT-Lung test, it was deemed appropriate to report the performance of the test in a clinical setting, where individuals at an increased risk of developing lung cancer were tested. Data from an audit of the first 1,612 samples run on the *Early*CDT-Lung test revealed that the performance of the test was as expected in a clinically relevant group of individuals at an increased risk of developing lung cancer, and the clinical results mirrored differences in the actual (as of August 2011) and calculated (Spitz model [22]) risk between the two groups. A difference in the gender proportion between the two clinical groups was noted; however, there are no reports of differences in autoantibody levels in individuals with lung cancer between genders [1]. Furthermore, a recent study of the demographics of normal individuals also showed no difference in autoantibody levels due to gender or ethnicity in a normal group [27].

Analysis of the performance of the *Early*CDT-Lung test in the clinical population dataset showed that the sensitivity of the test for lung cancer, reported in the optimization set, was maintained in the clinical setting, where at least 40% of the lung cancers had a positive test. Although the number of lung cancers in the audit was relatively small, both panels were successful in detecting early-stage disease.

The greatest impact seen with the new seven-AAb panel was the highly significant improvement in the specificity of the test in the clinical setting. While in the retrospective case–control set the improvement in the assay specificity resulted in a 30% reduction in false positives, in the prospective clinical audit data, the change to the seven-AAb panel resulted in a 44% reduction in the number of "false positive" tests. This is because in the clinical population, the specificity of the six-AAb panel was lower than expected at 82%, while the seven-AAb panel revealed a specificity of 90% (unadjusted for occult cancers), a level similar to that predicted from the optimization dataset. An individual predicted to be at an increased risk of lung cancer due to demographic risk factors including smoking history, gender, and age, and who then had a positive *EarlyCDT*-Lung test, would be at a higher risk for harboring lung cancer than predicted; with the introduction of the seven-AAb version of the test, this increase in risk is even greater.

The seven-AAb test with a specificity/sensitivity of 93%/41% in a high-risk population (e.g., prevalence of 2.4% [25]) has an overall accuracy of 92% compared to approximately 50% for CT [28]. The authors, however, view AAb technology and CT imaging as being complementary rather than competitive and that the presence of AAbs may provide an aid to early detection of lung cancer, particularly in early-stage disease which is potentially curable. This improved test may therefore prove useful in the management of high-risk individuals.

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Conflicts of interest GFH, AM, JA, CBPK, and IKM are employed by Oncimmune Ltd and LJP is employed by Oncimmune LLC. JFRR consults for and is a shareholder in Oncimmune Ltd. GHF is a shareholder and chairman of Oncimmune Ltd. CJC, WJ, and CR consult for Oncimmune Ltd.

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Clinical validation of an autoantibody test for lung cancer

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Background: Autoantibodies may be present in a variety of underlying cancers several years before tumours can be detected and testing for their presence may allow earlier diagnosis. We report the clinical validation of an autoantibody panel in newly diagnosed patients with lung cancer (LC).

Patients and methods: Three cohorts of patients with newly diagnosed LC were identified: group 1 ($n = 145$), group 2 ($n = 241$) and group 3 ($n = 269$). Patients were individually matched by gender, age and smoking history to a control individual with no history of malignant disease. Serum samples were obtained after diagnosis but before any anticancer treatment. Autoantibody levels were measured against a panel of six tumour-related antigens (p53, NY-ESO-1, CAGE, GBU4-5, Annexin 1 and SOX2). Assay sensitivity was tested in relation to demographic variables and cancer type/stage.

Results: The autoantibody panel demonstrated a sensitivity/specificity of 36%/91%, 39%/89% and 37%/90% in groups 1, 2 and 3, respectively, with good reproducibility. There was no significant difference between different LC stages, indicating that the antigens included covered the different types of LC well.

Conclusion: This assay confirms the value of an autoantibody panel as a diagnostic tool and offers a potential system for monitoring patients at high risk of LC.

Key words: autoantibodies, clinical validation, lung cancer, newly diagnosed patients

introduction

Lung cancer (LC) is the worldwide leading cause of cancer-related mortality [1]. Tobacco smoking is estimated to cause upwards of 90% of cases, and other recognised risk factors include passive smoking, occupational exposure, especially to asbestos and radon exposure [1]. Outcomes are substantially better with early localised disease compared with locally advanced and metastatic disease, with 5-year survival rates of 53%, 23.7% and 3.5%, respectively [2].

Although the latent period of LC in smokers is reported to be at least 20 years [1], ~85% of patients with LC remain undiagnosed until the disease is symptomatic and has reached an advanced stage [2]. At present, there is nothing to offer for early diagnosis, although ongoing clinical trials are investigating the use of spiral computed tomography (CT) in 'at-risk' individuals [3–12]. However, the radiation dose delivered and the substantial costs limit its widespread application as a screening procedure [13]. Furthermore, the high rate of false positives (as high as 50% in a prevalence round) [5] dictates that many individuals require follow-up examinations and a substantial proportion of individuals undergo unnecessary thoracotomy [14]. Application of a filter such as a blood-based marker to identify smokers at the highest risk for LC may improve the positive predictive value (PPV) of these screening tools [11, 15].

There is a considerable body of evidence documenting the presence of circulating antibodies to autologous cellular antigens [referred to as tumour-associated antigens (TAA)] in serum samples from patients with a variety of cancers, including LC [16–24]. Monitoring persons at increased risk of cancer for the presence of serum autoantibodies may allow earlier detection of the disease.

The panel of proteins selected for investigation comprised a number of well-recognised TAA, four of which (p53, NY-ESO-1, CAGE and GBU4-5) have been described by ourselves in a previous publication to induce the production of autoantibodies or immune biomarkers in LC [24]. In brief, p53 is a tumour suppressor gene, which is often mutated in cancer and to which autoantibodies were first described [25],

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autoantibodies to this protein have also been detected in some cases, even before the cancer diagnosis [26, 27]. NY-ESO-1 and CAGE are both cancer testis antigens whose expression has been described in a number of solid tumours [28, 29] and with GBU4-5, a protein of unknown function that encodes a DEAD box domain, have also been described as inducing autoantibodies in LC [24, 30, 31].

The remaining antigens SOX2, a member of the SOXB1 family of proteins that is described as inducing an autoantibody responses in small-cell lung cancer (SCLC) [32, 33], and Annexin I, a phospholipid-binding protein to which autoantibodies, have also been described [18].

The selection of these antigens was confirmed following screening of a panel of >20 potential antigens as being of greatest diagnostic utility for the diagnosis of all non-small-cell lung cancer (NSCLC) and SCLC cancer (C. Chapman, unpublished observations).

This manuscript reports the clinical validation set for these autoantibodies in the serum of patients with newly diagnosed LC (before any treatment) and matched controls.

patients and methods

patients

Findings from three separate groups of patients with newly diagnosed LC are reported. The third group is the final validation set where the data were run in a blinded manner. All patients with LC were as far as possible individually matched by gender, age and smoking history to a control individual with no previous history of malignant disease. In patients with LC, blood samples were obtained after diagnosis but before receiving any anticancer treatment. Demographic characteristics of the control versus the study population are given in the Appendix 1.

Group 1 comprised 145 patients with stage I/II LC (including NSCLC and SCLC) and 146 controls treated in centres in the United States and Russia. All subjects in this group were smokers; baseline patient characteristics are shown in Table 1. Group 2 comprised 241 patients with LC treated at a single centre in Germany as part of a collaborative study (Table 1). Tumour pathological information was available for the patients with LC, including tumour, node, metastasis staging and NSCLC histology (Table 2). In group 2, an additional 88 sera from unmatched individuals (25 normal and 63 with benign lung conditions) supplied by the single centre were analysed (Appendix 1).

Group 3 comprised 269 patients with LC treated at centres in the United States, UK and Ukraine (Table 1). This group was assembled to validate the calibration and control scheme for the autoantibody assay. Tumour pathological information was available for the patients with LC (Table 2). The timeline for collection of samples from patients is shown in supplemental Table S1 (available at *Annals of Oncology* online).

Table 1. Lung cancer patient characteristics

	Group 1 (n = 145)	Group 2 (n = 241)	Group 3 (n = 269)
Median age, years (range)	66 (41–87)	63 (28–87)	65 (38–87)
Patients >60 years, n (%)	96 (66.2)	140 (58.1)	171 (63.6)
Gender, n (%)			
Male	81 (55.9)	172 (71.4)	199 (74.0)
Female	64 (44.1)	69 (28.6)	70 (26.0)
Smoking history, n (%)			
Current	145	0	132 (49.1)
Previous	0	0	76 (28.3)
Never	0	0	24 (8.9)
Not determined	0 (0.0)	241 (100.0)	37 (13.8)

Serum samples in group 1 were evaluated for autoantibodies against p53, NY-ESO-1, CAGE and GBU4-5. Serum samples in groups 2 and 3 were evaluated for autoantibodies against the same four antigens plus Annexin 1 and SOX2. In groups 2 and 3, samples from patients with cancers, matched normals, benign lung disease and control sera for the assay were interspersed in the order samples were assayed so that any batch effects would be spread over all sample types. The laboratory staff running the assay was blinded to the disease state of individual samples. Group 2, therefore, was a validation set for the results seen in group 1 for four of the antigens (i.e. p53, NY-ESO1, CAGE and GBU4-5) with the added value of Annexin 1 and SOX2. Group 3 validated a calibrated and controlled assay on the whole panel of six antigens.

autoantibody assay

Autoantibodies were determined by a quality-controlled, semi-automated indirect enzyme-linked immunosorbent assay in which samples were allowed to react with a titration series of antigen concentrations. All liquid handling steps were carried out using an automated liquid handling system. Briefly, purified recombinant antigens were diluted to provide a semi-log titration series for each antigen from 160 to 1.6 nM [34]. Control antigens consisting of the purified BirA or NusA tags were also included to allow subtraction of the signal due to nonspecific binding to bacterial contaminants. Antigen dilutions were adsorbed to the surface of microtitre plate wells in phosphate buffer at room temperature. After washing in phosphate-buffered saline containing 0.1% Tween 20 (pH 7.6), microtitre plates were blocked with a gelatine-based blocking buffer. Serum samples (diluted 1 in 110 in a blocking buffer) were then added to the plates and allowed to incubate at room temperature with shaking for 90 min. Following incubation, plates were washed and horseradish peroxidase-conjugated rabbit anti-human IgG (Dako, Glostrup, Denmark) was added. After a 60-min incubation, the plates were washed and 3,3#,5,5#-tetramethylbenzidine was added. Colour formation was allowed to proceed for 15 min before the optical density (OD) of each well was determined spectrophotometrically at 650 nm [35].

Calibration standards of known potency are not available for assays to measure autoantibodies against TAAs. Therefore, a calibration system was devised which utilised fluids drained from pleural or ascitic cavities of patients with LC [36]. The calibration system was only evaluated for group 3 samples. A reportable dilution range for each antigen, giving acceptable calibration precision, was determined at 7.5%–92.5% of the upper asymptote of the average calibration curve, equivalent to ~5.0 natural log reference units (RU). These data were used to construct a calibration curve of OD versus log dilution to which a four-parameter model plot was fitted [37]. The background-corrected OD value for each unknown sample was then converted to a calibrated log RU.

Samples were judged to be positive if they fulfilled two criteria—i.e. they showed a dose response to the antigen titration series and the measured autoantibody signal to one or more of the antigens was above the accepted cut-off set for that antigen assay. The autoantibody signal for a sample was defined as above the cut-off when the result was greater than the calculated cut-off for the control population at either of the two highest points on the titration curve. All assays were carried out as two replicates and the mean value taken as the overall assay measurement.

optimisation of assay cut-offs

A specificity of 90% was selected in order to produce a test which could be used for early detection in a high-risk population and which would be health economically viable. For all groups, cut-offs based on mean + 3

Table 2. Tumour stage and histology according to gender

	Group 1 (n = 145)		Group 2 (n = 241)		Group 3 (n = 255[a])	
	Male (n = 81)	Female (n = 64)	Male (n = 172)	Female (n = 69)	Male (n = 188)	Female (n = 67)
Tumour type, n (%)						
NSCLC	71 (87.7)	52 (81.3)	125 (72.7)	46 (66.7)	141 (75.0)	41 (61.2)
SCLC	10 (12.3)	12 (18.8)	47 (27.3)	23 (33.3)	47 (25.0)	26 (38.8)
NSCLC stage, n (%)						
I	41 (57.7)	40 (76.9)	0 (0.0)	0 (0.0)	71 (50.4)	15 (36.6)
II	30 (42.3)	12 (23.1)	0 (0.0)	1 (2.2)	42 (29.8)	11 (26.8)
III	0 (0.0)	0 (0.0)	38 (30.4)	11 (23.9)	13 (9.2)	1 (2.4)
IV	0 (0.0)	0 (0.0)	63 (50.4)	25 (54.3)	1 (0.7)	2 (4.9)
Unknown	0 (0.0)	0 (0.0)	24 (19.2)	9 (19.6)	14 (9.9)	12 (29.3)
NSCLC histology, n (%)						
Squamous	16 (22.5)	5 (9.6)	38 (30.4)	4 (8.7)	78 (55.3)	10 (24.4)
Adenocarcinoma	16 (22.5)	13 (25.0)	37 (29.6)	19 (41.3)	44 (31.2)	23 (56.1)
Large cell	2 (2.8)	0	4 (3.2)	2 (4.3)	5 (3.5)	0 (0.0)
Bronchoalveolar	1 (1.4)	18 (34.6)	0 (0.0)	0 (0.0)	1 (0.7)	5 (12.2)
Tubular adenocarcinoma	0	0	0	0	2 (1.4)	0
Not determined	4 (5.6)	12 (23.1)	46 (36.8)	21 (45.6)	9 (6.4)	1 (2.4)
Other	32 (45.1)	4 (7.7)	0	0	2 (1.4)	2 (4.9)
SCLC stage, n (%)						
Limited SCLC	0	0	21 (44.7)	6 (26.1)	14 (29.8)	15 (57.7)
Extensive SCLC	0	0	19 (40.4)	14 (60.9)	8 (17.0)	3 (11.5)
Not determined	10 (100.0)	12 (100.0)	7 (14.9)	3 (13.0)	25 (53.2)	8 (30.8)

[a]Tumour histology and stage data available for 255 of the 269 patients comprising group 3.
NSCLC, non-small-cell lung cancer; SCLC, small-cell lung cancer.

standard deviations (SDs) of the normal population were used. In addition, for groups 2 and 3, the cut-offs were optimised using a Monte Carlo direct search method [38] to find a set of antigen-specific cut-offs yielding the maximum sensitivity for the fixed specificity of 90%.

For a set of possible cut-offs for the six panel antigens chosen by Monte Carlo sampling over the feasible range, the specificity/sensitivity was first estimated from the data. This was carried out 100 000 times. All combinations with a specificity of ~90% were then extracted and the combination yielding the maximum sensitivity used. This is a process dependent on assay conditions and when new batches of proteins, or new types of protein, are introduced to the panel, new cut-offs will have to be calculated.

To support the quoted specificity/sensitivity panel results, the area under the curve (AUC) and standard error (SE) for the respective receiver operating characteristic (ROC) curve was calculated for each group. The ROC curve was constructed by calculating the specificity and sensitivity of the test for a succession of deviations from the original cut-offs, with the same deviation for each antigen in the panel.

adjustment for LCs in the control populations

The cut-offs are best set by comparing the results in a group of patients with known LC and a group of high-risk individuals (e.g. smokers and ex-smokers) who are known to not have the disease. However, the latter population is difficult to identify since the CT screening studies have clearly shown there are a percentage of smokers/ex-smokers who at any one time are 'harbouring' an asymptomatic LC. In the prevalence round, the percentage of undiagnosed occult cancers has been reported to be between 0.5% and 2.7% in heavy smokers, while in incidence rounds, it has been reported to be up to 2.3% [3–12]. For this reason, adjusted specificity and sensitivity values assuming some degree of occult LCs in the control populations were also calculated.

The method used to calculate and adjust for the presence of undiagnosed cancers in the controls used LC prediction models for which the most important predictors are age, current smoking status and smoking history, and family history of smoking-related cancers [39].

results

autoantibody expression

In group 1, autoantibodies to four antigens (p53, NY-ESO-1, CAGE and GBU4-5) were measured as raw OD values. Using cut-offs based on mean + 3 SDs gave a sensitivity of 36% with a specificity of 91% (50 of the 137, 8 unassessable). The sensitivities and specificities for each of these four antigens and the reproducibility of these assays have been reported elsewhere [35]. The sensitivity and specificity of the panel was similar for males and females. The ROC curve AUC was 0.71 (SE = 0.03).

In group 2, autoantibodies to six antigens (p53, NY-ESO-1, CAGE, GBU4-5 plus Annexin 1 and SOX2) were measured as raw OD values, with cut-offs based on mean + 3 SDs producing sensitivity and specificity values of 34% (80 of the 234, 7 unassessable) and 91%, respectively. Again, individual sensitivities and specificities for these six antigens have been reported elsewhere [35]. Using individually optimised cut-offs for each antigen, the overall sensitivity was 39% (33%–45%) (91 of the 234), with a specificity of 89%. In an at-risk population of 20 LCs per 1000 population, this would result in a PPV of 7.2% (i.e. 1 in 13.9 persons with a positive test would have a LC) and a negative predictive value (NPV) of 98.6%. The ROC curve AUC was 0.63 (SE = 0.03).

Of the 88 unmatched sera received from the group 2 centre, 8 of the 88 (9%) were positive, of which none of the 25 (0%) normal sera had raised autoantibodies, while 8 of the 63 (13%) of individuals with benign lung disease had raised autoantibodies. Follow-up data could only be obtained for one of these eight individuals who was found to have developed a gastric cancer, giving a specificity of at least 89% (55 of the 62).

In group 3, autoantibodies to the same six antigens as group 2 were measured as raw OD values and converted into calibrated RU. Using the ODs and applying cut-offs based on mean + 3 SDs gave a sensitivity of 32% (85 of the 269) with a specificity of 91%. Using RU values with individually optimised cut-offs for each antigen, the sensitivity was 37% (31%–43%) (100 of the 269), with a specificity of 90%. In an at-risk population of 20 LCs per 1000 population, this would result in a PPV of 7.0% (i.e. 1 in 14.3) and an NPV of 98.6%. The ROC curve AUC was 0.64 (SE = 0.02).

Individual antigen sensitivity and specificity are shown in supplemental Table S2 (available at *Annals of Oncology* online).

adjustment for occult LCs within the control population

Adjustment generated specific cut-offs for each antigen for the different methods. The sensitivities for each antigen for a fixed specificity of 90% are shown for the unadjusted and adjusted method in Table 3. The most conservative estimate for adjusted sensitivity is 40%, which in an at-risk group of 20 LCs per 1000 population would give a PPV of 7.5% (i.e. 1 in 13.3) and an NPV of 98.7%.

effect of patient and disease characteristics on autoantibody assay sensitivity and specificity

The calibrated group 3 dataset with an unadjusted sensitivity and specificity of 37% and 90%, respectively, was used to assess whether patient characteristics, tumour type or stage gave rise to significant variation in the specificity/sensitivity (Figure 1). Statistical comparison of subgroups with remaining controls demonstrated no significant difference in sensitivity according to patient gender, smoking status and age or tumour type or stage ($P > 0.10$). There was also no significant difference in sensitivity between those NSCLC tumours where the subtype was known and those where it was unknown.

discussion

This report confirms a validated assay for the detection of autoantibodies to selected cancer-associated antigens in the peripheral blood. The value of a test for early cancer detection is usually defined via a number of related parameters, including sensitivity, specificity, PPV and NPV. A percentage of smokers/ex-smokers are 'harbouring' an asymptomatic LC at any one time. Even with the most conservative estimation of occult LCs, the panel of autoantibodies can identify 40% of primary LCs, including early stage of disease, with a specificity of 90% against age-matched, gender-matched and smoking history-matched controls. The specificity was similar (at least 89%) for patients with benign disease.

Table 3. Comparison of performance[a] before and after adjustment for the presence of undiagnosed occult cancers in the control population

Adjustment method		Group 2	Group 3
Unadjusted	Sensitivity	39% (91/234)	37% (100/269)
	Specificity	89% (207/232)	90% (242/269)
Occult cancer rate (5%)	Sensitivity	42% (99/234)	40% (108/269)
	Specificity	90% (197/220)	90% (230/255)
Occult cancer rate (11%)	Sensitivity	46% (108/234)	43% (115/269)
	Specificity	89% (184/206)	90% (214/238)

[a]Sensitivity for specificity of 90% 6 1%, based on optimised cut-offs for individual antigens.

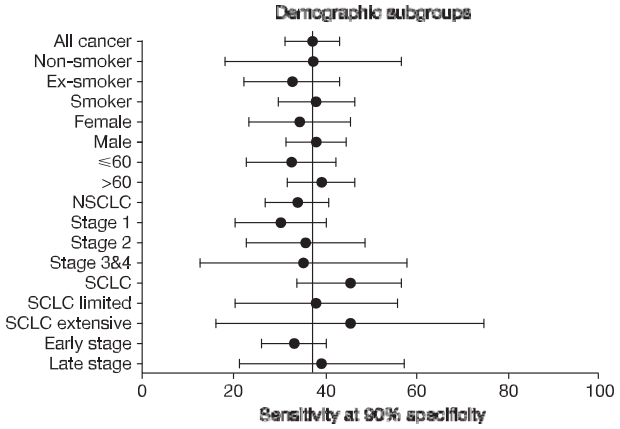

Figure 1. Forest plot showing the sensitivity at a fixed 90% specificity by patient demographics, tumour characteristics and lung cancer stage. Line shows unadjusted sensitivity of 37% (all stages of cancers) in group 3 (n = 269). NSCLC, non-small-cell lung cancer; SCLC, small-cell lung cancer.

Autoantibodies to p53 [26, 27, 40, 41], NY-ESO-1 [30, 31], CAGE [29, 42], GBU4-5 [31], Annexin 1 [16, 18, 43] and SOX2 [44] have all been shown to be capable of inducing autoantibodies in patients with LC. The data in this manuscript further confirm the value of a panel of autoantibodies over a single autoantibody assay [19, 23, 24, 35]. Recent publications have reported autoantibodies to a natural form of Annexin 1 [43] and other antigens (e.g. 14-3-3 theta [43, 45] and LAMR1 [43]), which are elevated in LC and up to 1 year before clinical diagnosis. The combination of 14-3-3 theta, Annexin 1 and LAMR1 gave an AUC on a combined ROC curve of 0.73. While these results were based on a research assay, it is possible that adding 14-3-3 theta and/or LAMR1 to the current panel might increase the sensitivity.

Group 3 data confirm that there was no significant difference between different stages of LC, although due to sample size the confidence intervals were sometimes wide. Further evaluation of the data was, therefore, carried out by comparing early-stage (stage I/II NSCLC plus limited SCLC) with late-stage (stage III/IV NSCLC plus extensive SCLC) disease, which again showed no difference. The presence of such a signal in early-stage disease is precisely what would be expected of an *in vivo* amplification signal such as the humoural immune response.

This is in contrast to cancer-associated antigens, which are markers of tumour burden and not useful for the early detection or screening of breast [46, 47] or colorectal cancer [48, 49].

Previous publications [16–24, 50] have highlighted the potential value of a panel of autoantibodies for the early detection of cancer. Using a panel of antigens, autoantibodies have been reported up to 5 years before screening CT scans [22] in LC and up to 4 years before screening mammography in young women at increased risk [21, 23]. Other authors have highlighted individual autoantibodies such as p53 autoantibodies detected before diagnosis of cancer in smokers with chronic obstructive pulmonary disease [27] or in patients with asbestosis [41]. In the latter publication, the average lead time (time from first positive sample to diagnosis) was 3.5 years (range 1–12 years). There are similar publications on other single autoantibodies [45, 51, 52]. These findings all indicate the induction of autoantibodies happening relatively early in the process of carcinogenesis.

This panel assay is the first to show reproducible results with a calibration and control system and offers a potential system for monitoring a population at high risk of LC, either alone or in conjunction with imaging modalities (e.g. CT). The similar sensitivities and specificities measured for these three datasets and with different batches of proteins utilised emphasise the robustness of these autoantibody assays and also confirm the value of a panel of autoantibodies over a single autoantibody assay.

At a fixed 90% specificity, the sensitivity of 40% is a conservative estimate of the performance of the assay both in terms of estimating the level of clinically occult LCs (supplemental Table S2, available at *Annals of Oncology* online) and also the sensitivity reported for SCLC ($n = 73$) in group 3. The latter is lower than the 55% sensitivity and 90% specificity, which the authors will report in a larger consecutive series ($n = 242$) from a single centre (C. J. Chapman, A. J. Thorpe, A. Murray et al., unpublished data).

The sensitivity of 40% with a specificity of 90% are similar to mammography in high-risk young women [53], while the incidence of LCs in heavy smokers is at least three times the incidence of breast cancer in a typical cohort of high-risk young women [5, 7]. Therefore, in terms of absolute number of cancers, this test should detect more LCs for every 1000 high-risk persons tested than screening mammography would detect breast cancers in a high-risk group of young women, even if mammography were 100% sensitive rather than its current 40% [53]. This has to be seen in the context of a disease (i.e. LC), which has a mortality rate between 85% and 95%. By way of contrast, annual CT in the Mayo CT screening trial had a specificity of 49% (with a sensitivity of 67%) in the prevalence round. In an at-risk group of 20 of the 1000, CT gave a PPV of 2.5% (i.e. 1:40) and an NPV of 98.7%. The autoantibody test with a sensitivity of 40% and a specificity of 90% would have a PPV of 7.5% (i.e. 1 in 13.3) and an NPV of 98.7% in a similar-risk group.

While such comparisons serve to highlight the potential value of an autoantibody test for LC that has a specificity of 90%, the authors envisage the autoantibody technology and imaging as being complementary.

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disclosure

CJC and JFRR are consultants to Oncimmune Ltd, a University of Nottingham spinout company and JFRR holds stock. AM and GH are full-time employees of Oncimmune Ltd. CR holds stock option and is also a consultant to Oncimmune Ltd. WCW is the scientific advisor for Oncimmune Ltd. GH is an employee of Oncimmune Ltd. GHF is the Chairman of Oncimmune Ltd and holds stock. ACB holds stock and options in Oncimmune Ltd and has a significant conflict.

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appendix 1

demographic characteristics of the control versus the study population

A total of 655 lung cancer (LC) sera (476 were from patients with non-small-cell lung cancer, 165 with small-cell lung cancer, 1 lung sarcoma and 13 of unknown histology) were compared directly with 655 normal sera, which were analysed as controls. In addition, sera from 88 unmatched individuals (25 normal and 63 with benign lung conditions) supplied by the group 2 centre were analysed as controls to check the positivity rate in known benign lung disease. Samples were obtained, with full informed consent, at different sites. Controls for patients in group 1 were matched on the basis of gender and age (64 years). As all subjects in this group were smokers, pack-year matching was attempted, but a tight match was prohibited by lack of information. There were 81 males and 64 females in the LC group and 83 males and 62 females in the

control group. The median age (range) of the LC patients and controls were 66 (41–87) and 66 (41–87) years, respectively. In group 2, there were 172 males and 69 females in the LC group and 171 males and 69 females in the control group. The median age (range) of the LC patients and controls were 63 (28–87) and 63 (28–87) years, respectively. Controls for group 2 were selected from a prospective collection of blood samples taken from a larger sample set of a normal population in the Midlands of England. Patients with LC were initially matched to controls on the basis of gender, age (63 years) and smoking history. In <5% of cases, these criteria could not be met, so a choice had to be made to either extend the age-match criteria or ignore the gender-match stipulation. Since the authors have never observed a significant gender difference, age and smoking history were given priority over gender. In 37 LC patients, the exact smoking history was unknown, and in a further four patients, age matching was >3 years.

The group 2 centre also supplied 88 unmatched samples from individuals who were either thought to be normal (n = 25) or have a range of benign lung diseases (n = 63), including mass/nodule (n = 3), autoimmune lung disease (n = 10), chronic obstructive pulmonary disease/emphysema (n = 2), benign pleural effusion (n = 2), allergic/inflammatory/infective conditions (n = 25) (e.g. allergic alveolitis, Wegner's granulomatosis, asthma, sarcoid, vasculitis, Dessler's syndrome, mycoplasmosis, tuberculosis) and nonspecified lung disorders (n = 21). A set of individually matched controls for this group of LC patients was selected from a prospective collection of blood samples taken from a normal population in the UK. Controls were matched on the basis of gender and age. With the exception of one patient who was matched to 64 years, controls were matched to patient age 62 years. Smoking history was not known for the patients with LC, so controls were simply selected from a population of smokers and ex-smokers.

In group 3, there were 199 males and 70 females in the LC group and 187 males and 82 females in the control group. The median age (range) in the LC and control groups was 65 (38–87) and 65 (38–86) years, respectively.

The matched controls in group 3 were collected as part of a larger sample set of the normal population (n = 766) in the Midwest United States and demographic data included ethnicity. Evaluation of calibrated reference unit (RU) for autoantibody expression demonstrated that when controlled for age, there was no significant difference between ethnic groups [Caucasians (n = 614), African Americans (n = 108), Hispanics (n = 27) and Native Americans (n = 17)] in terms of calibrated RUs (data not shown). There was a further set of samples from 125 normal individuals who were located in Florida and age matched, gender matched and smoking history matched to a similar number of the controls in the Midwest United States (n = 125). The Florida samples were part of another larger prospective collection of sera from the normal population. Comparison of the 125 samples from each of these two normal populations from different geographic and ethnic backgrounds showed no significant difference in the calibrated RU values for any of the six antigens (data not shown).

Signal stratification of autoantibody levels in serum samples and its application to the early detection of lung cancer

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ABSTRACT

Background: Further signal stratification for the EarlyCDT®-Lung test should facilitate interpretation of the test, leading to more precise interventions for particular patients.

Methods: Samples were measured for the presence of autoantibodies to seven tumor-associated antigens (TAAs) (p53, NY-ESO-1, CAGE, GBU4-5, SOX2, MAGE A4, and HuD). In addition to the current test cut-offs (determined using a previously reported Validation case-control sample set, set A; n=501), new high and low cut-offs were set in order to maximize the test's positive and negative predictive values (PPV and NPV, respectively). All three sets of cut-offs were applied to two confirmatory datasets: (I) the case-control set B (n=751), and (II) Population-derived set C (n=883), and all three datasets combined (n=2,135).

Results: For the Validation dataset, cancer/non-cancer positivity for current cut-offs was 41%/9% (PPV =0.109, 1 in 9). The high positive stratum improved this to 25%/2% (PPV =0.274, 1 in 4). The low negative stratum improved this to 8%/23% (NPV =0.990, 1 in 105). This provides a 25-fold difference in lung cancer probability between the highest and lowest groups.

The test performs equally well in subjects who fulfilled the entry risk criteria for the National Lung Screening Trial (NLST) and subjects who did not meet the NLST criteria.

Conclusions: The *Early*CDT®-Lung test has been converted to a four-stratum test by the addition of high and low sets of cut-offs: patients are thus stratified into four risk categories. This stratification will enable personalization of subsequent screening and treatment programs for high risk individuals or patients with lung nodules.

KEYWORDS Lung cancer; autoantibody (AAb); tumor-associated antigen; risk stratification

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Introduction

The technical and clinical validation of an autoantibody (AAb) assay for the early detection of lung cancer (*Early*CDT®-Lung) has recently been described (1-3). In a series of case-control studies, where the cases were newly diagnosed lung cancer patients, circulating AAbs to a panel of tumor-associated antigens (TAAs) were measured in serum samples. Validation of the 7 AAb panel showed that *Early*CDT®-Lung can, with a specificity of 93%, detect elevated levels of AAbs in peripheral blood samples for up to 41% of all primary lung cancers (3). In combination with imaging techniques, the test is now commercially available to assist clinicians in the early detection of lung cancer in a high-risk population.

Currently a single test threshold ("cut-off") for each AAb measured in the panel classifies the samples into two strata, i.e., positive or negative for AAbs associated with lung cancer. This two-stratum test yields a useful binary classification, but given the range of intervention options available to the clinician, refinement of the result is desirable. A four-stratum test is therefore now proposed with additional sets of low and high cut-offs to classify the results into high positive, positive, negative and low negative strata indicating relatively very high, high, low and very low levels of AAbs, respectively; the level of AAbs measured relates to the probability of lung cancer (i.e.,

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Table 1. Result categories for the four-stratum *Early*CDT®-Lung test.

Block	Rule	Result	Risk[a]
1	At least one AAb > H	High positive	Very high
2	All AAbs < H, but at least one >C	Positive	High
3	All AAbs < C, but at least one > L	Negative	Low
4	All AAbs < L	Low negative	Very low

AAb, autoantibody; L, low cut-off; C, current cut-off; H, high cut-off; [a], Risk (i.e., probability) of having a lung cancer at the time of the test.

risk of having the disease) (Table 1). This allows more refined intervention for different sub-groups of patients.

Materials and methods

Assay procedure

AAbs to seven TAAs (p53, NY-ESO-1, CAGE, GBU4-5, SOX2, MAGE A4, and HuD) were measured using *Early*CDT®-Lung (Oncimmune USA LLC, De Soto, KS, USA), a commercially available blood test based on indirect enzyme-linked immunosorbent assay (ELISA) methods, that uses microtiter plates coated with semi-log serial dilutions of recombinant antigens (1). AAb levels were measured as optical density units, background-corrected and then converted to calibrated reference units (RU). Each patient serum sample was assayed in duplicate on each plate and a titration curve obtained for each antigen. A sample was declared positive if there was a clear titration curve, and if the RU at either of the two highest points on the titration curve was above its respective cut-off for at least one antigen. Quality control samples were interspersed in the sample order.

Patient samples

Three separate sets of serum samples were used in this work, two case-control sets described previously, and a new population-based set. All patients provided written informed consent for their samples to be used in this study.

Sample set A (Validation case-control)

This set comprised 235 patients with lung cancer from UK, US, Ukraine, and Russia (obtained at or just after diagnosis) representing 87% of the cancers in a previously published dataset (Group 3, n=269) (2) for which enough volume was available to complete the panel of seven AAbs (3). There were 179 non-small-cell lung cancers (NSCLCs, 76%), 53 small-cell lung cancers (SCLCs, 23%), and three others (1%). The controls, all recruited in the US from the general population, came from the same sample set and comprised 266 healthy volunteers with no history of cancer, 235 of whom were matched to the cases by age, gender, and smoking history (2).

Sample set B (Post-validation case-control)

Four groups of patients (Groups 1-4) with newly diagnosed lung cancer, but prior to treatment, plus controls matched by gender, age (±4 years) and smoking history (as far as possible), were combined into a single dataset, as previously reported (4). Group 1 comprised 32 cases with SCLC from a single UK center and Group 2 comprised 161 cases from multiple European centers. Controls (±4 years) came from a prospective collection of cancer-free smokers in the Midlands of England and the Midwest of America. Group 3 comprised 120 cases from a single center in Vancouver, Canada, matched to 113 high-risk lung-cancer-free controls. Group 4 comprised 23 cases matched to 109 controls. The total sample set comprised 336 lung cancer cases, including 301 NSCLC (90%) and 35 SCLC (10%), and 415 normal control sera. The incomplete matching in Groups 2 to 4 was mainly due to controls being excluded if they had been used for another group or if sample volume was insufficient.

Sample set C (Population)

This set comprised 847 commercially-derived samples collected consecutively between November 2010 and February 2012 from individuals deemed by their clinicians as being at high risk of developing lung cancer. Clinical follow-up information available through a prospective audit is known for all these individuals of whom 36/847 (4.3%) were diagnosed [using computed tomography (CT) and/or biopsy] with lung cancer within 6 months after taking the test. Ethnicity was known for 823 (97%) of patients.

Derivation of cut-offs

The current test cut-offs divide the samples into two strata, positive or negative, corresponding to high and low lung cancer risk respectively, so as to maximize the sensitivity for a specificity of about 90% (2). As previously reported (2,3), the specificity was also adjusted for the presence of an estimated small number of undiagnosed cancers in the control group. In the Population dataset, individuals were defined as 'cancer-free' if a lung cancer diagnosis was not obtained within six months after testing (manuscript in preparation).

Using sample set A, a new set of high cut-offs, splitting the two-stratum positives, was derived by adding a multiple of

Dataset	Cut-offs	Two-stratum		Four-stratum			
		Positivity (C/N) (PPV, 1/PPV)	Negativity (C/N) [NPV, 1/(1-NPV)]	Positivity (C/N) (PPV, 1/PPV)		Negativity (C/N) [NPV, 1/(1-NPV)]	
		Current positive	Current negative	High positive	Positive	Negative	Low negative
NLST[a]	Incidence	43/36	73/379	31/9	12/27	56/270	17/109
(116C/415N)	Unadjusted	37%/9%	63%/91%	27%/2%	10%/7%	48%/65%	15%/26%
	2.7%	(0.106, 1 in 9)	(0.981, 1 in 53)	(0.255, 1 in 4)	(0.042, 1 in 24)	(0.980, 1 in 50)	(0.985, 1 in 66)
Non-NLST[b]	Incidence	103/89	225/854	61/23	42/66	198/595	27/259
(328C/943N)	Unadjusted	31%/9%	69%/91%	19%/2%	13%/7%	60%/63%	8%/28%
	2.7%	(0.085, 1 in 12)	(0.979, 1 in 49)	(0.175, 1 in 6)	(0.048, 1 in 21)	(0.974, 1 in 39)	(0.992, 1 in 121)
[c]P-value		0.91	0.03	0.56	0.37	0.005	0.22
[d]P-value		C 0.26/N 0.65		C 0.03/N 0.91			

[a]Individuals who met the NLST criteria for lung cancer screening; [b]Individuals who did not meet the NLST criteria for lung cancer screening; [c]Association of Cancer status and NLST eligibility using χ^2 test; [d]Association of EarlyCDT-Lung positivity and NLST eligibility using χ^2 test. C, cancers; N, normals (cancer-free controls).

the standard deviation of the distribution of controls to the current cut-off for each autoantibody to optimize specificity and sensitivity to yield a high positive predictive value (PPV). Similarly, a set of low cut-offs, splitting the two-stratum negatives, was derived by subtracting multiples of standard deviations to yield a high negative predictive value (NPV). The main calculations were performed assuming a cancer prevalence of 2.7% (2), but tabulation for 4%, being the typical five-year lung cancer risk for an average smoker, was also carried out. All analyses were carried out using SAS® (Version 9.1.3, Cary, NC, USA).

Statistical analysis

The new cut-offs were applied to all datasets, separately and combined, thus sorting patients into four strata on the basis of their *Early*CDT®-Lung AAb levels (Table 1). To check the consistency of the classification, the percentages of samples within the new strata were compared across datasets for cases and controls separately using Fisher Exact tests (5).

Using the specificity and sensitivity for each stratum, the PPV and NPV were then derived using the number of samples in the stratum versus their complement, the samples not in the stratum. A continuous estimate of five-year demographic risk based on gender, age and, where available, smoking history was also derived using a modified version of the Spitz model (6). The demographic factors and staging on the stratification were investigated using multinomial modelling (SAS®, Proc GENMOD and Proc FREQ).

A further analysis compared subjects who could or could not be classified according to the main National Lung Screening Trial (NLST) trial inclusion criteria, i.e., age (55-74 years old) and smoking history (≥30 pack years and quit <15 years ago)(7). In the combined dataset (n=1,802), 531 subjects met the NLST criteria (29%) 116 of whom (22%) were lung cancers, while 1,271 did not (non-NLST) (71%) 328 of whom (26%) were lung cancers (Tables 2,3). There were more under-age subjects than over-age for sets A & B: 21% under-age, 68% NLST and 13% over-age. For sample set C (population set), the figures were 31%, 58% and 11%, respectively. Also, Non-NLST subjects had all smoked less. Two associations were tested (χ^2 tests): (I) between cancer status and NLST eligibility for each separate test stratum, and (II) between *Early*CDT-Lung positivity and NLST eligibility for cancers and non-cancer subjects separately.

Results

Patient samples

The patient demographics were summarized for the sample sets A, B & C separately (Table 4, full details in Table 5). Demographics for sample sets A & B were representative of patients with lung cancer, with more males than females, age ranging from 23 to 90 years and more than half of patients being at least 60 years old. In sample set C (the Population dataset), however, there was a higher percentage of females, suggesting that females are more likely to be proactive about their health, and with a median age of the cases about 10 years older than for the controls. The pattern of smoking was similar over all three datasets, although with a tendency for the cases to be current smokers and controls to be ex-smokers. Mean demographic risk in sample set C was higher for cases than for controls, reflecting the differences noted above.

Analysis using the current and newly defined test cut-offs

For both the current two-stratum test and the new four-stratum

Table 3. Incidence, stratified by NLST Inclusion: case-control and population set separate.

					Positive		Low negative
Case-control	NLST[a]	Incidence 38/17	60/15	29/5	9/12	47/117	13/33
(409C/617N)	(98C/167N)	Unadjusted 39%/10%	61%/90%	30%/3%	9%/7%	48%/70%	13%/20%
		2.7% (0.096, 1 in 10)	(0.981, 1 in 54)	(0.215, 1 in 5)	(0.034, 1 in 29)	(0.981, 1 in 54)	(0.982, 1 in 55)
	Non-NLST[b]	Incidence 95/41	216/409	56/14	39/27	191/304	25/105
	(311C/450N)	Unadjusted 31%/9%	69%/91%	18%/3%	13%/6%	61%/68%	8%/23%
		2.7% (0.085, 1 in 12)	(0.979, 1 in 48)	(0.138, 1 in 7)	(0.055, 1 in 18)	(0.975, 1 in 41)	(0.991, 1 in 106)
[a]P-value		0.92	0.11	0.51	0.19	0.02	0.20
[b]P-value		C 0.13/N 0.69		C 0.02/N 0.78			
Population	NLST[a]	Incidence 5/19	13/229	2/4	3/15	9/153	4/76
(35C/741N)	(18C/248N)	Unadjusted 28%/8%	72%/92%	11%/2%	17%/6%	50%/62%	22%/31%
		2.7% (0.091, 1 in 11)	(0.979, 1 in 47)	(0.160, 1 in 6)	(0.071, 1 in 14)	(0.978, 1 in 45)	(0.980, 1 in 51)
	Non-NLST[b]	Incidence 8/48	9/445	5/9	3/39	7/291	2/154
	(17C/493N)	Unadjusted 47%/10%	53%/90%	29%/2%	18%/8%	41%/59%	12%/31%
		2.7% (0.118, 1 in 8)	(0.984, 1 in 62)	(0.309, 1 in 3)	(0.058, 1 in 17)	(0.981, 1 in 53)	(0.990, 1 in 97)
[c]P-value		0.47	0.01	0.92	0.26	0.07	0.09
[d]P-value		C 0.24/N 0.35		C 0.54/N 0.79			

[a]Individuals who met the NLST criteria for lung cancer screening; [b]Individuals who did not meet the NLST criteria for lung cancer screening; [c]Association of Cancer status and NLST eligibility using χ^2 test; [d]Association of *Early*CDT-Lung positivity and NLST eligibility using χ^2 test. C, cancers; N, normals (cancer-free controls).

Table 4. Brief summary of demographics.

Dataset	Controls/cases			
	Males	Median age	Smoker	Ex-smoker
Set A	70%/73%	65/65	35%/46%	54%/29%
Set B	64%/65%	62/67	19%/52%	57%/33%
Set C	36%/42%	60/70	45%/50%	41%/44%

test, using the cut-offs derived from sample set A, the sensitivity, specificity, PPV and NPV were calculated for each dataset and for all datasets combined (Table 6, full details in Table 7). For convenience the NPV is also presented in its reciprocal form (1 in X), i.e., the probability of cancer given a negative result.

Sample set A

The two-stratum test gave a cancer/normal positivity of 41%/9% (PPV=0.109, 1 in 9), and the four-stratum test high positive stratum improved this to 25%/2% (PPV =0.274, 1 in 4) (Tables 6,7). The two-stratum test also gave a cancer/normal negativity of 59%/91% (NPV =0.982, 1 in 57), and the four-stratum test low negative stratum improved this to 8%/23% (NPV=0.990, 1 in 105). For the demographic split, no difference (5% level) between strata was seen for gender (P=0.99), age category (P=0.053) or smoking status (P=0.37), similarly for staging profile (P=0.16).

Sample set B

The two-stratum test gave a cancer/normal positivity of 30%/10% (PPV =0.076, 1 in 13), and the high positive stratum improved this to 17%/4% (PPV =0.113, 1 in 9). The two-stratum test also gave a cancer/normal negativity of 70%/90% (NPV =0.979, 1 in 47), and the low negative stratum improved this to 12%/22% (NPV =0.985, 1 in 65) (Table 6). No difference between strata was seen for gender (P=0.88), age category (P=0.62) or smoking status (P=0.57), similarly for staging profile (P=0.21).

Sample set C

The two-stratum test gave a cancer/normal positivity of 36%/9% (PPV =0.103, 1 in 10), and the high positive stratum improved this to 19%/2% (PPV =0.226, 1 in 4). The two-stratum test also gave a cancer/normal negativity of 64%/91% (NPV =0.981, 1 in 53), and the low negative stratum improved this slightly to 17%/31% (NPV = 0.985, 1 in 67) (Table 6). Again, no difference between strata was seen for gender (P=0.20), age category (P=0.07) or smoking status (P=0.51), similarly for the proportion of Caucasians (P=0.22). There were too few cancers to investigate staging.

Combined set

The three datasets were pooled into a single Combined dataset, with 607 cases and 1,492 controls. The two-stratum test gave a cancer/normal positivity of 34%/9% (PPV =0.094,

Table 5. Summary of demographics by dataset.

	Sample set A dataset		Sample set B dataset		Sample set C (Population) dataset	
	Controls N=266	Cases N=235	Controls N=415	Cases N=336	Controls (Cancer-free) N=811	Cases N=36
Tumor type, n (%)						
NSCLC	n/a	179 (76%)	n/a	301 (89%)	n/a	32 (88%)
Stage I		79 (34%)		170 (51%)		16 (44%)
Stage II		48 (20%)		45 (13%)		5 (13%)
Stage III		14 (6%)		44 (13%)		8 (22%)
Stage IV		3 (1%)		21 (6%)		2 (6%)
Stage unknown		35 (15%)		21 (6%)		1 (3%)
SCLC	n/a	53 (23%)	n/a	35 (11%)	n/a	2 (6%)
Limited SCLC		23 (10%)		6 (2%)		1 (3%)
Extensive SCLC		7 (3%)		26 (8%)		1 (3%)
Stage unknown		23 (10%)		3 (1%)		0 (0%)
Type unknown	n/a	3 (1%)	n/a	0 (0%)	n/a	2 (6%)
Gender, n (%)						
Male	185 (70%)	171 (73%)	265 (64%)[a]	218 (65%)	290 (36%)	15 (42%)
Female	81 (30%)	64 (27%)	148 (36%)	118 (35%)	521 (64%)	21 (58%)
Age						
Age, median [min-max]	65 [38-86]	65 [42-85]	62 [23-87]	67 [23-90]	60 [35-85]	70 [49-85]
Age, mean +/- sem	64 +/- 0.6	65 +/- 0.6	62 +/- 0.5	65 +/- 0.6	61 +/- 0.4	70 +/- 1.4
Race, n (% of known)						
Caucasian	n/r	n/r	n/r	n/r	721 (91%, n=789)	29 (85%, n=34)
African-American	n/r	n/r	n/r	n/r	37 (5%, n=789)	4 (12%, n=34)
Smoker						
Yes, n (%)	93 (35%)	108 (46%)	78 (19%)	175 (52%)	361 (45%)	18 (50%)
Pk-yrs, mean +/- sem	36 +/- 2 (n=92)	31 +/- 2 (n=86)	31 +/- 3 (n=69)	32 +/- 2 (n=147)	41 +/- 1 (n=346)	45 +/- 6 (n=18)
Risk (Modified Spitz)	3.0 (0.3)	3.3 (0.3)	3.1 (0.3)	3.4 (0.2)	2.4 (0.1)	5.9 (0.6)
Ex, n (%)	144 (54%)	67 (29%)	237 (57%)	112 (33%)	331 (41%)	16 (44%)
Pk-yrs, mean +/- sem	32 +/- 3 (n=105)	38 +/- 4 (n=37)	31 +/- 2 (n=223)	39 +/- 2 (n=72)	40 +/- 2 (n=315)	52 +/- 9 (n=16)
Risk (Modified Spitz)	3.8 (0.2)	3.7 (0.3)	3.4 (0.1)	5.1 (0.3)	3.7 (0.1)	5.8 (0.4)
No, n (%)	29 (11%)	24 (10%)	99 (24%)	43 (13%)	117 (14%)	2 (6%)
Risk (Modified Spitz)	0.2 (0.02)	0.1 (0.02)	0.1 (0.01)	0.2 (0.01)	0.1 (0.01)	0.1 (0.02)
Unknown	0 (0%)	36 (15%)	1 (0%)	6 (2%)	2 (0%)	0 (0%)

[a]Gender unknown for two subjects. n/a, not applicable; n/r, information not recorded; NSCLC, non-small-cell lung carcinoma; SCLC, small-cell lung carcinoma; sem, standard error of the mean; Pk-yrs, Pack-years. Rounding applied to percentages to ensure 100% totals.

Table 6. Summary of PPV and NPV for two-stratum and four-stratum test.

Dataset	Two-stratum test		Four-stratum test			
	Positive PPV	Negative NPV	High positive PPV	Positive PPV	Negative NPV	Low negative NPV
Set A	10.9% (1 in 9)	1.8% (1 in 57)	27.4% (1 in 4)	5.5% (1 in 18)	2.0% (1 in 49)	1.0% (1 in 105)
Set B	7.6% (1 in 13)	2.1% (1 in 47)	11.3% (1 in 9)	5.4% (1 in 19)	2.3% (1 in 43)	1.5% (1 in 65)
Set C	10.3% (1 in 10)	1.9% (1 in 53)	22.6% (1 in 4)	6.3% (1 in 16)	2.1% (1 in 47)	1.5% (1 in 67)
Combined	9.4% (1 in 11)	2.0% (1 in 51)	19.3% (1 in 5)	5.4% (1 in 18)	2.3% (1 in 43)	1.1% (1 in 90)

PPV, positive predictive value; NPV, negative predictive value, in its reciprocal form, i.e., 1-NPV. Based on a population lung cancer prevalence of 2.7%.

Table 7. Incidence with PPV and NPV for two-stratum and four-stratum formats.

							) [NPV, 1/(1-NPV)]
							Low negative[f]
Sample set A	Incidence	97/25	138/241	60/5	37/20	119/179	19/62
(235C/266N)	Unadjusted	41%/9%	59%/91%,	25%/2%	16%/7%	51%/68%	8%/23%
	2.7%	(0.109, 1 in 9)	(0.982, 1 in 57)	(0.274, 1 in 4)	(0.055, 1 in 18)	(0.980, 1 in 49)	(0.990, 1 in 105)
	4.0%	(0.155, 1 in 6)	(0.974, 1 in 38)	(0.361, 1 in 3)	(0.080, 1 in 12)	(0.970, 1 in 33)	(0.986, 1 in 70)
Sample set B	Incidence	99/41	237/374	56/15	43/26	196/284	41/90
(336C/415N)	Unadjusted	30%/10%	70%/90%	17%/4%	13%/6%	58%/68%	12%/22%
	2.7%	(0.076, 1 in 13)	(0.979, 1 in 47)	(0.113, 1 in 9)	(0.054, 1 in 19)	(0.977, 1 in 43)	(0.985, 1 in 65)
	4.0%	(0.111, 1 in 9)	(0.968, 1 in 32)	(0.161, 1 in 6)	(0.078, 1 in 13)	(0.966, 1 in 29)	(0.977, 1 in 44)
Sample set C	Incidence	13/71	23/740	7/15	6/56	17/492	6/248
(36C/811N)	Unadjusted	36%/9%	64%/91%	19%/2%	17%/7%	47%/60%	17%/31%
	2.7%	(0.103, 1 in 10)	(0.981, 1 in 53)	(0.226, 1 in 4)	(0.063, 1 in 16)	(0.979, 1 in 47)	(0.985, 1 in 67)
	4.0%	(0.147, 1 in 7)	(0.972, 1 in 35)	(0.305, 1 in 3)	(0.091, 1 in 11)	(0.969, 1 in 32)	(0.978, 1 in 45)
Combined	Incidence	209/137	398/1,355	123/35	86/102	332/955	66/400
(607C/1492N)	Unadjusted	34%/9%	66%/91%	20%/2%	14%/7%	55%/64%	11%/27%
	2.7%	(0.094, 1 in 11)	(0.980, 1 in 51)	(0.193, 1 in 5)	(0.054, 1 in 18)	(0.977, 1 in 43)	(0.989, 1 in 90)
	4.0%	(0.135, 1 in 7)	(0.971, 1 in 34)	(0.265, 1 in 4)	(0.079, 1 in 13)	(0.966, 1 in 29)	(0.983, 1 in 60)
Sample set A	Adjusted	41%/7%	59%/93%	25%/0.04%	16%/7%	51%/69%	8%/24%
(235C/266N)	2.7%	(0.145, 1 in 7)	(0.983, 1 in 58)	(0.947, 1 in 1.06)	(0.061, 1 in 16)	(0.980, 1 in 50)	(0.991, 1 in 109)
	4.0%	(0.203, 1 in 5)	(0.974, 1 in 39)	(0.964, 1 in 1.04)	(0.089, 1 in 11)	(0.970, 1 in 34)	(0.986, 1 in 73)
P-value[g]				0.03/0.16	0.49/0.80	0.12/0.013	0.13/0.001

[a]Includes all samples above current cut-off; [b]Includes all samples below current cut-off; [c]Includes all samples above high cut-off; [d]Includes only samples between current and high cut-off; [e]Includes only samples between current and low cut-off; [f]Includes all samples below low cut-off; [g]P-value for comparison of percentages across Sample set A, B & C datasets (cancers/normals separately) using χ^2 test. C, cancers; N, normals (cancer-free controls); NPV, negative predictive value; PPV, positive predictive value. PPV and NPV calculated for a population with a prevalence of 2.7% or 4.0% using unrounded specificity/sensitivity values.

1 in 11) and the high positive stratum improved this to 20%/2% (PPV =0.193, 1 in 5). The two-stratum cut-offs also gave a cancer/normal negativity of 66%/91% (NPV =0.980, 1 in 51) and the low negative stratum improved this to 11%/27% (NPV =0.989, 1 in 90) (Table 6). Further analysis showed clear consistency of the EarlyCDT-Lung risk profile across age decades (Tables 8,9).

Comparison across datasets

The positivity percentages were consistent across the three datasets for both cases and controls (Table 6). For the low negative stratum, the percentage of negatives in sample set C 'controls' (31%) was higher than for the other two datasets (23% and 22% respectively, P=0.001), which could reflect the higher number of younger cancer-free individuals in the population sample set C. Even despite the age difference, some dataset-to-dataset variation is to be expected, and the difference was not great. This consistency confirmed that the new sample set A cut-offs were directly applicable to sample sets B & C.

Risk analysis

In standard demographic models (e.g., Spitz) (6), risk increases with age and degree of smoking. To assess the independence of demographic risk and EarlyCDT-Lung result, a single threshold was applied to demographic risk to classify samples into low and high risk. This allowed 2×2×2 tables of positivity (demographic risk, EarlyCDT-Lung result, cancer status) to be compiled, bearing in mind the matching in the case-control sets. No evidence was found for a departure from independence (proportionality) of demographic risk and EarlyCDT-Lung.

The modification of the personalized continuous demographic risk by the four-stratum test is also under investigation. Based on DLR (diagnostic likelihood ratio) calculations (8) for typical cases (e.g., middle-aged moderate smokers), going from a positive result in the two-stratum test to a high positive result in the four-stratum test changed the risk increase from 4.3- to 12.7-fold. Similarly, going from a two-stratum negative result to a four-stratum low negative result changed the risk decrease from 1.5- to 2.9-fold.

Table 8. Age by *Early*CDT-Lung risk stratum, combined dataset (Cancers).

Stratum	Age group				Total
	20-49	50-59	60-69	70-90	
Very low risk	2 (5%)	16 (12%)	28 (14%)	20 (9%)	66 (11%)
Low risk	34 (78%)	74 (53%)	94 (47%)	130 (57%)	332 (55%)
High risk	2 (5%)	18 (13%)	29 (15%)	37 (16%)	86 (14%)
Very high risk	5 (12%)	31 (22%)	47 (24%)	40 (18%)	123 (20%)
Total	43	139	198	227	607

Table 9. Age by *Early*CDT-Lung risk stratum, combined dataset (Controls).

Stratum	Age group				Total
	20-49	50-59	60-69	70-90	
Very low risk	58 (33%)	139 (30%)	128 (26%)	75 (21%)	400 (27%)
Low risk	98 (57%)	281 (60%)	324 (66%)	252 (71%)	955 (64%)
High risk	14 (8%)	38 (8%)	27 (5%)	23 (6%)	102 (7%)
Very high risk	4 (2%)	8 (2%)	15 (3%)	8 (2%)	35 (2%)
Total	174	466	494	358	1,492

Percentages calculated within column. Test for an association between stratum profile within age.

EarlyCDT-Lung and NLST criteria

There was no evidence that the cancer rate differed between NLST and Non-NLST cohorts at the positive end of the test (Table 2). At the negative end, there were some small differences, but these were not consistent across sample sets A & B (more cancers in the Non-NLST cohort) and sample set C (Population dataset) (more cancers in the NLST cohort). Statistical significance was generally only seen when the table frequencies were high, and in fact, the differences were not large in the NPV estimates.

There was also little evidence for a difference between NLST and Non-NLST cohorts in their positivity profile. The only comparison significant at 5% was for the sample sets A & B cancers in the four-stratum test where there were more high positives in the NLST cohort (30%) than in the Non-NLST (18%) (Table 3), but this finding was not repeated in sample set C (Population dataset).

Discussion

Improvements in diagnostic test sensitivity and specificity, and hence PPV and NPV, facilitate clinical intervention decisions. This report confirms that the addition of high and low cut-offs to *Early*CDT®-Lung enables stratification of patients into very high risk for lung cancer, with improved PPV, or very low risk, with improved NPV.

Three lung cancer case-control sets were assessed. The case demographics were representative of patients with lung cancer: a predominance of males, more than half of patients >60 years of age, and over half the patients with early-stage lung cancer (i.e., NSCLC stages 1 or 2 or limited SCLC).

For the high positive stratum the specificity was set at 98%. In sample set A, this lowered the sensitivity from 41% for the positive stratum to 25%, but overall the PPV was greatly increased from 10.9% (1 in 9) to 27.4% (1 in 4). Similarly, for the low negative stratum the NPV increased from 98.2% (1 in 57) to 99.0% (1 in 105). The cost for this improvement is reduced performance for the two intermediate strata; for the positive stratum the PPV fell to 5.5% (1 in 18), whilst for the negative stratum the NPV fell to 98.0% (1 in 49).

Importantly, the consistency of performance when applied to sample sets B and C was found to be excellent (Tables 6,7). These data suggest that the *Early*CDT®-Lung measurements may provide a continuous variable in terms of lung cancer risk. We term this the Occurrence Score™ and it is under development.

There was no evidence for an association between demographic factors and *Early*CDT-Lung strata. The analysis clearly suggested that *Early*CDT®-Lung is adding to demographic risk independently.

The varied origin of the sample sets supports the general applicability of the results. Nodule data was not available for the case-control datasets. In the Population dataset, however, a positive *Early*CDT®-Lung result did add to the risk of a lung nodule being cancer (manuscript in preparation). The described AAb technology and CT imaging are potentially additive

rather than competitive since the presence of AAbs provides an opportunity for early detection of lung cancer, even in early-stage disease, and may therefore be useful in the management of high-risk individuals. Thus, for example, combining a low negative *Early*CDT®-Lung result with a negative CT scan would lead to a very high NPV (manuscript in preparation).

Finally, this study compared the *Early*CDT-Lung strata with whether or not patients met the entry criteria for the NLST study. Only 65% of participants in the Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial (PLCO) who developed lung cancers met the NLST criteria (9), and in another recent US study of early stage lung cancer patients (n=267) fewer than half met the criteria and would not be covered under current screening paradigms (10). In our analysis of *Early*CDT-Lung, little evidence was found that the cancer rate differed between NLST and Non-NLST cohorts, indicating that *Early*CDT-Lung provides similar risk stratification for these cohorts. Thus we can now identify individuals initially deemed at a risk lower than the NLST criteria whose risk after *Early*CDT-Lung is equivalent to the entry criteria for the NLST. This provides a rationale for identification and CT screening of individuals who fall outside the NLST criteria.

Conclusions

*Early*CDT-Lung is recommended as a tool for physicians to assess a patient's probability of lung cancer thereby facilitating the early detection of lung cancer. By applying two additional cut-offs, we have converted the test to a four-stratum version to allow further stratification of patients into different risk categories. This enhanced stratification can be used on a population that fulfills the NLST criteria to identify super high risk sub-groups. In addition, we have shown that *Early*CDT-Lung can increase the risk estimates for certain Non-NLST patients, and bring them into the NLST range, thus facilitating more appropriate intervention for such patients.

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BRIEF REPORT

Autoantibody Signature Enhances the Positive Predictive Power of Computed Tomography and Nodule-Based Risk Models for Detection of Lung Cancer

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ABSTRACT

Introduction: The incidence of pulmonary nodules is increasing with the movement toward screening for lung cancer by low-dose computed tomography. Given the large number of benign nodules detected by computed tomography, an adjunctive test capable of distinguishing malignant from benign nodules would benefit practitioners. The ability of the *Early*CDT-Lung blood test (Oncimmune Ltd., Nottingham, United Kingdom) to make this distinction by measuring autoantibodies to seven tumor-associated antigens was evaluated in a prospective registry.

Methods: Of the members of a cohort of 1987 individuals with Health Insurance Portability and Accountability Act authorization, those with pulmonary nodules detected, imaging, and pathology reports were reviewed. All patients for whom a nodule was identified within 6 months of testing by *Early*CDT-Lung were included. The additivity of the test to nodule size and nodule-based risk models was explored.

Results: A total of 451 patients (32%) had at least one nodule, leading to 296 eligible patients after exclusions, with a lung cancer prevalence of 25%. In 4- to 20-mm nodules, a positive test result represented a greater than twofold increased relative risk for development of lung cancer as compared with a negative test result. Also, when the "both-positive rule" for combining binary tests was used, adding *Early*CDT-Lung to risk models improved diagnostic performance with high specificity (>92%) and positive predictive value (>70%).

Conclusions: A positive autoantibody test result reflects a significant increased risk for malignancy in lung nodules 4 to 20 mm in largest diameter. These data confirm that *Early*CDT-Lung may add value to the armamentarium of the practitioner in assessing the risk for malignancy in indeterminate pulmonary nodules.

Keywords: Autoantibodies; Lung cancer; Pulmonary nodules; CT scanning; Risk models

Introduction

More than 1.5 million individuals in the United States have lung nodules identified annually, including 63,000 who receive a new lung cancer diagnosis within 2 years.[1,2] This number is growing given the increasing number of computed tomography (CT) scans being performed since the National Lung Screening Trial reported that annual CT screening reduced lung cancer

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mortality by 20% as compared with chest radiography.[3] The problem remains, however, that more than 50% of CT-screened patients have at least one noncalcified nodule, with more than 96% of nodules larger than 4 mm being false-positives.[3,4] Furthermore, there is a high false-positive rate and morbidity associated with biopsy or resection of benign nodules.[2,3,5] Therefore, a critical need exists for an adjunctive test to help evaluate the malignancy potential and reduce the false-positive rate. *Early*CDT-Lung (Oncimmune Ltd., Nottingham, United Kingdom) detects the presence of serum autoantibodies (AAbs) to a panel of lung cancer–associated antigens using an indirect enzyme-linked immunosorbent assay method.[6,7] A sample is positive if at least one AAb is elevated above a predetermined cutoff. Test specificity was improved in November 2010 by changing from a six-AAb panel to the current seven-AAb panel.[8] Test performance in routine clinical practice for approximately 1600 patients with unknown nodule status was assessed previously, showing 87% specificity, 41% sensitivity, and a 5.4-fold relative risk of lung cancer in cases with a positive test result.[9] We demonstrate how the test can assist in evaluation of the malignancy of pulmonary nodules in the clinic with or without lung cancer risk models.

Methods

Patients

A cohort of 1987 Health Insurance Portability and Accountability Act–authorized patients was tested in routine practice between May 2009 and December 2012 by using *Early*CDT-Lung as described previously.[8–10] For

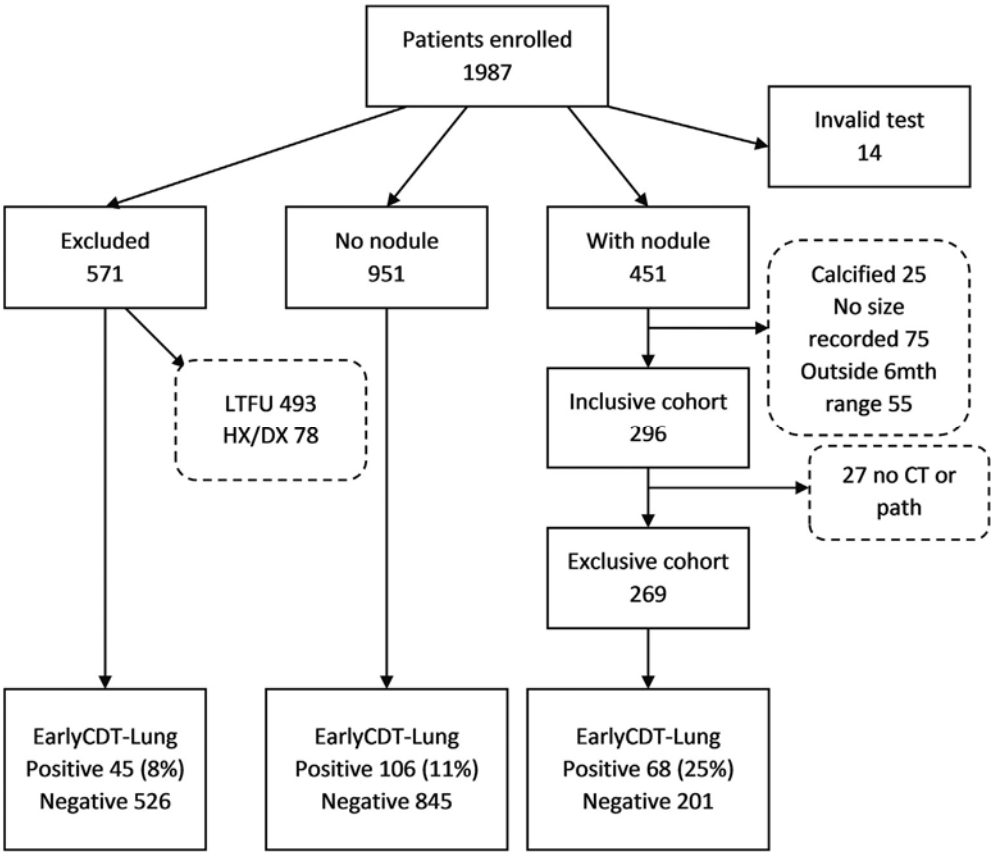

Figure 1. Patient cohorts in the clinical registry of nodule status. Of 1987 patients enrolled, 585 (29%) were excluded either on the basis of being lost to follow-up (LTFU) for all reasons, including practices closed, patients changing practices, and physicians moving practices (n¼493), or on the basis of having received a confirmed cancer diagnosis (Dx) other than lung cancer, a history (Hx) of cancer (n¼78), or an invalid autoantibody test result (n ¼14). Of the remaining 1402 patients (71% of the 1987), 451 (339 without cancer and 112 with cancer) had a pulmonary nodule(s) reported, whereas the remaining 951 either did not have a nodule detected by computed tomography (CT), did not have an available CT, were reported as unknown by the physician, or had a CT but with unknown results. In the 451 patients with a nodule, the nodule was calcified in 25 individuals, 75 nodules had no clear size information, and a further 55 were outside the 6-month time window, leaving 296 patients (221 without cancer and 75 with cancer) for the inclusive cohort and 269 (217 without cancer and 52 with cancer) for the exclusive cohort. The *Early*CDT-Lung positivity rates for the excluded, no-nodule, and with-nodule groups were 8%, 11%, and 25%, respectively, with the comparison of the excluded and no-nodule groups being of borderline significance (*p*¼ 0.05) and thus giving no clear evidence of bias.

Table 1. Summary of Demographics of Patients with Nodules (Inclusive and Exclusive Data Sets)

Characteristic	Inclusive	Exclusive
Data set size	296	269
Sex (M-to-F)	145:151	133:136
Age, median (range), y	66 (30–89)	66 (30–89)
Smoker (current, ex, no)[a]	121, 128, 43	110, 113, 42
6AAb, 7AAb	114, 182	103, 166
Lung cancers[b]	75	52
Tumor type (NSCLC, SCLC, others)	56, 4, 15	44, 2, 6
Staging (early, late, unknown)	34, 21, 20	28, 17, 7

[a]Four patients had incomplete smoking data.
[b]Number of cancers diagnosed at up to 6 months' follow-up.
M, male; F, female; AAb, autoantibody.

this study CT imaging reports from within 6 months of the AAb test were assessed for the presence of pulmonary nodules. The size of the largest noncalcified nodule was recorded. Patients who had invalid AAb tests, were lost to follow-up, or had a history of cancer were excluded. PET scan data were generally unavailable.

Although most cancer cases were confirmed by pathology reports, some were diagnosed solely on imaging reports. To reflect this, the analysis considered two cohorts defined by their eligibility criteria (Fig. 1).

Inclusive Cohort. The clinician's diagnosis was accepted even if CT or pathology reports were not available. This corresponds most closely to the commercial setting. The inclusive cohort retains as many cancers as possible to enhance statistical power.

Exclusive Cohort. Starting with the inclusive cohort, only those members with data from actual CTs and pathology reports on file, as opposed to office visits or physician phone interviews, were accepted. These criteria correspond most closely to ideal clinical practice.

Statistical Analysis

A *model-free analysis* summarizing the association between nodule size (<4 mm, 4–20 mm, and :::20 mm),

Table 2. Comparison of Test Performance Characteristics in the Inclusive and Exclusive Cohorts by Nodule Size

Inclusive cohort (n ¼ 296)[a]

Category	Positive AAb				Negative AAb					
Size (mm)	LC	Not LC	Total	PPV	LC	Not LC	Total	RR	DLRp	*p* Value
<4	1	8	9	—	0	18	18	—	—	—
4 to <20	14	35	49	29%	20	127	147	2.1 (1.2–3.8)	1.9 (1.2–3.1)	0.028
:::20	15	3	18	83%	25	30	55	1.8 (1.3–2.6)	4.1 (1.3–13.0)	0.006
Overall	30	46	76	39%	45	175	220	1.9 (1.3–2.8)	1.9 (1.3–2.8)	0.002

Exclusive cohort (n ¼ 269)[b]

Category	Positive AAb				Negative AAb					
Size (mm)	LC	Not LC	Total	PPV	LC	Not LC	Total	RR	DLRp	*p* Value
<4	1	8	9	—	0	17	17	—	—	—
4 to <20	11	35	46	24%	12	124	136	2.7 (1.3–5.7)	2.2 (1.3–3.6)	0.018
:::20	10	3	13	77%	18	30	48	2.1 (1.3–3.3)	3.9 (1.2–12.9)	0.014
Overall	22	46	68	32%	30	171	201	2.2 (1.3–3.5)	2.0 (1.3–3.0)	0.003

Exclusive, 7AAb cohort (n ¼ 166)[c]

Category	Positive AAb				Negative AAb					
Size (mm)	LC	Not LC	Total	PPV	LC	Not LC	Total	RR	DLRp	*p* Value
<4	0	5	5	—	0	13	13	—	—	—
4 to <20	6	14	20	30%	9	73	82	2.7 (1.1–6.8)	2.5 (1.1–5.4)	0.071
:::20	8	2	10	80%	14	22	36	2.1 (1.2–3.4)	4.4 (1.0–18.4)	0.032
Overall	14	21	35	40%	23	108	131	2.3 (1.3–3.9)	2.3 (1.3–4.1)	0.010

Note: p Values for comparing *Early*CDT-Lung results by malignant and nonmalignant nodules were calculated using Fisher's exact test; 95% binomial confidence intervals are shown. Nodule size coding: small ¼ 3 mm; sub cm ¼ 5 mm; tiny ¼ 2 mm; <4 ¼3 mm; 3–4 ¼ 4 mm; <10 ¼5 mm; 17–20 ¼ 19 mm.
[a]Overall specificity ¼ 79%, sensitivity ¼ 40%, PPV ¼ 39%, NPV ¼ 80%. Comparison of positivity between size categories (excluding <4 mm) (p¼ 0.95).
[b]Overall specificity ¼ 79%, sensitivity ¼ 42%, PPV ¼ 32%, NPV ¼ 85%. Comparison of positivity between size categories (excluding <4 mm) (p¼ 0.53).
[c]Overall specificity ¼ 84%, sensitivity ¼ 38%, PPV ¼ 40%, NPV ¼ 82%. Comparison of positivity between size categories (excluding <4 mm) (p¼ 0.77).
AAB, autoantibody; LC, lung cancer; PPV, positive predictive value; RR, relative risk; and DLRp, positive diagnostic likelihood ratio, all defined in the text (see Statistical Analysis section).

Table 3. Calibration of Risk Models versus Observed Cancers in the Registry—Inclusive Cohort (n ¼ 296)

				Mean Predicted Cancer Risk			
Size	n	Observed Cancers	Cancer Rate	GOULD	BROCK	MAYO	Mayo Study
<4 mm	27	1	4%	15%	—[a]	5%	0%
4 to <8 mm	75	6	8%	24%	0%	8%	1%
8 to <20 mm	121	28	23%	39%	8%	24%	21%
:::20	73	40	55%	77%	40%	70%	33%
All	296[a]	75	25%	42%	13%	29%	17%
C[2] test[b]				$p < 0.001$	$p < 0.001$	p ¼ 0.54	$p < 0.01$

[a]BROCK not available for <4 mm.
[b]Chi-square test for comparison of audit-observed versus expected cancers for each model and Mayo study.

cancer diagnosis, and test positivity was performed. Diagnostic performance metrics were calculated for the inclusive, exclusive, and exclusive (7AAb panel only) cohorts. Pretest risk was calculated by applying size thresholds above which a patient would be deemed to have a positive result (nodules-only). Posttest risk (positive predictive value [PPV]) was calculated by adding the AAb test result using a "both-positive rule" (i.e., positive for both size threshold and AAb test result). The curve for PPV versus nodule size threshold was compared with that for the combined test (nodules þ AAb). Finally, the observed nodule distribution was compared with the Mayo study[4] to confirm similarity.

A *risk-model analysis* was applied using the inclusive cohort. The pretest cancer risk was estimated using three published nodule-based models: (1) the GOULD model, which is based on Department of Veterans Affairs data[11]; (2) the BROCK model, which was developed on the basis of pan-Canadian study data[12]; and (3) the MAYO model, which is based on Mayo study data.[13,14] The calibration of these models over nodule size was examined by comparing observed and predicted cancer rates using C[2] goodness-of-fit tests.

The additivity of the AAb test to the models was assessed for 4-mm to 20-mm nodules, which are the most relevant for early detection. The receiver operating characteristic curve for each model was formed by varying the risk threshold above which a patient was deemed positive and calculating specificity and sensitivity at each threshold (model only). Then, at each threshold the data were split by AAb test result and a new curve was formed by applying the both-positive rule (Model þ AAb). The sensitivity observed at fixed specificity values was compared across the two curves. Plots of the respective PPVs were also created.

Results

Cohorts

From the original cohort (n¼ 1987), exclusions led to 296 patients (221 without cancer and 75 with cancer) for the inclusive cohort and 269 (217 without cancer and 52 with cancer) for the exclusive cohort (Table 1 and Supplementary Table 1 for the six-AAb and seven-AAb panels).

Model-Free Analysis

The nodule distribution was most similar to that in the Mayo study (Supplementary Fig. 1). For the exclusive

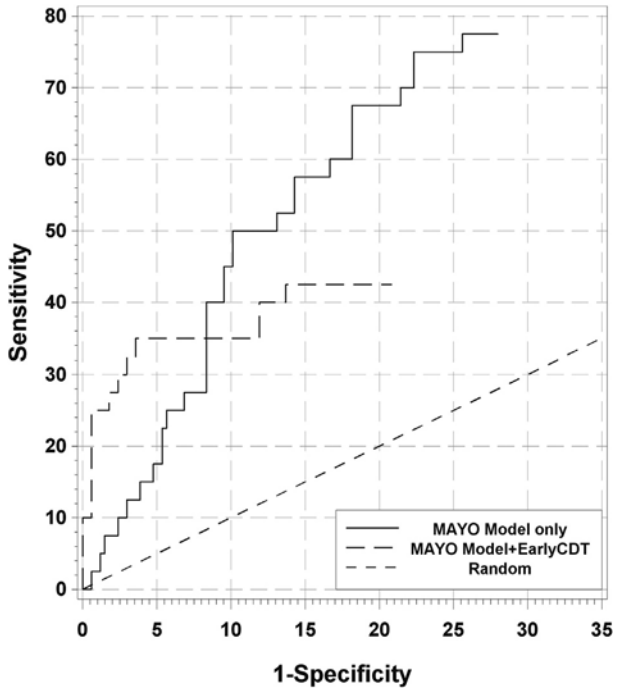

Figure 2. Partial receiver operating characteristic curves for the MAYO model and with *Early*CDT-Lung added using the both-positive rule. Curves are shown for the MAYO model only (*black line*); the MAYO model plus *Early*CDT-Lung (*dashed line*); the proportional line, which is the theoretical line if *Early*CDT-Lung was added to the MAYO model in a strictly proportional (independent) manner (*dotted line*); and the random line of no diagnostic discrimination (*thin black line*). Below approximately 8% on the *x* axis (92% specificity) the combined model and autoantibody test show improved sensitivity at the same specificity. The proportional line follows the observed line quite closely (inclusive cohort, 4 mm–20 mm [n ¼ 208]).

Table 4. Example Using a Specificity of 97%

Model	Model Only			Model þ AAb			
MAYO		Negative	Positive		Either Negative	Both Positive	Total
	No LC	163	5	No LC	163	5	168
	LC	35	5	LC	27	13	40
	Total	198	10	Total	190	18	208
Risk		60%			20%		
Spec, Sens		97%, 13%			97%, 33%		
PPV		50%			72%		
RR		2.8 (1.4–5.6)			5.1 (3.2–8.0)		
DLRp		4.2 (1.3–13.8)			10.9 (4.1–28.9)		

Note: The effect of adding *Early*CDT-Lung at a reduced risk level (60%–20%) is to reclassify eight cancers from false-negative to true positive. This gave high performance statistics: PPV ¼ 72%, RR 5.1 ¼ and DLRp 10.9 ¼

AAb, autoantibody; LC, lung cancer; Risk, risk threshold; Spec, specificity; Sens, sensitivity; PPV, positive predictive value; RR, relative risk with 95% confidence interval; DLRp, positive diagnostic likelihood ratio with 95% confidence interval.

cohort, 22 of 68 of patients with a positive test result (32%) and 30 of 201 of patients with a negative test result (15%) had lung cancer, giving a relative risk of 2.2 (95% confidence interval [CI]: 1.3–3.5, PPV ¼ 32%) (Table 2). For the seven-AAb test the relative risk increased to 2.3 (95% CI: 1.3–3.9, PPV ¼ 40%). For the 4-mm to 20-mm group, the relative risk was 2.7-fold (95% CI: 1.3–5.7), equating to an increase in absolute risk from 13% to 24%, and for the 20-mm group, the relative risk was 2.1-fold (95% CI: 1.3-3.3), which equates to an increase in absolute risk from 46% to 77% (Supplementary Fig. 2).

p Values for comparing *Early*CDT-Lung results by malignant and nonmalignant nodules were calculated using Fisher's exact test. The 95% binomial confidence intervals are shown.

Risk Model Analysis

For the inclusive cohort, the GOULD and BROCK models, respectively, overestimated and underestimated the risk relative to the actual rates observed ($p<0.001$). The MAYO estimates were closest to those observed (p 0.54) (Table 3).

For nodules in the 4- to 20-mm range, the receiver operating characteristic curves for the models show that as specificity surpassed 90%, the sensitivity declined to 30% and less (Fig. 2 for MAYO and Supplementary Figures 3 and 4 for GOULD and BROCK, respectively). When *Early*CDT-Lung was added, the sensitivity was increased at the high-specificity end (left-hand side). More precisely, for a fixed risk threshold, when a biomarker test was added using the both-positives rule, the specificity increased and the sensitivity decreased. For the MAYO model, for example, as the risk threshold moved from 35% to 60%, the specificity for the model-only case increased from 88% to 97% and the sensitivity decreased from 50% to 13%

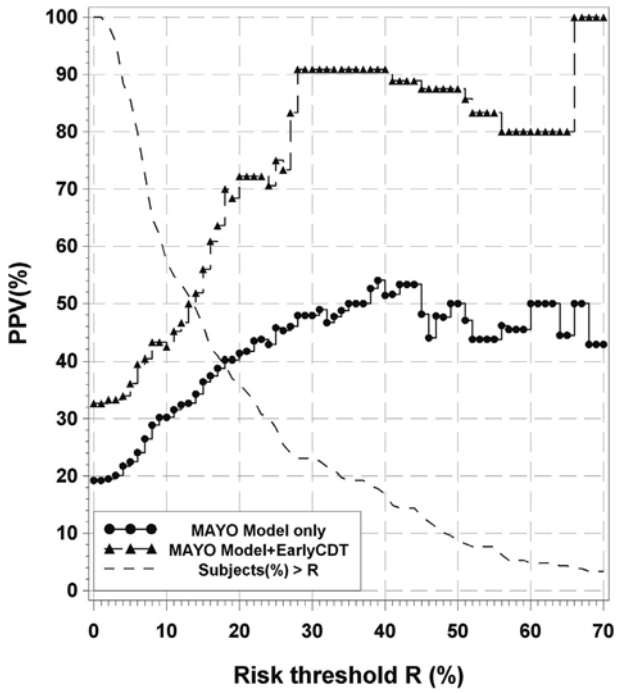

Figure 3. Plot of positive predictive value (PPV) versus risk threshold (R) for the MAYO model. Curves are shown for the MAYO model only (*black line with dots*) and the MAYO model plus *Early*CDT-Lung (*gray dashed line with triangles*). Also shown is the plot of the percentage of subjects with an individual risk below R on the *x* axis (*black dashed line*). Adding *Early*CDT-Lung improves the PPV over the whole range. For a chosen risk threshold, a patient is risk model–positive if his or her calculated individual risk is greater than the threshold. The procedure could be as follows: Choose R (30%, for example) and then read off the model-only PPV for patients with a risk higher than R (48% in this example) and then with an *Early*CDT-Lung–positive result added (91%). Finally, read off the percentage of patients in the population with a risk higher than R (23%). The number of false-positive results is reduced at the expense of fewer cancers detected. So choose the value of R giving the most useful performance (inclusive cohort, 4 mm–20 mm [n ¼ 208]).

Table 5. Example Using a Risk Threshold of 30% with the MAYO Model

Model	Model Only			Model þ AAb			
MAYO		Negative	Positive		Either Negative	Both Positive	Total
	No LC	143	25	No LC	167	1	168
	LC	17	23	LC	30	10	40
	Total	160	48	Total	197	11	208
Risk		30%			30%		
Spec, Sens		85%, 58%			99%, 25%		
PPV		48%			91%		
RR		4.5 (2.6–7.7)			6.0 (4.1–8.7)		
DLRp		3.9 (2.5–6.1)			42.0 (5.5–318.9)		

Note: The effect of adding *Early*CDT-Lung was to eliminate virtually all false-positive results (25 down to 1) at the expense of losing just more than half of the cancers (23 down to 11). This gave high performance statistics: PPV ¼ 91%, RR ¼ 6.0, and DLRp ¼ 42.0.
AAb, autoantibody; LC, lung cancer; Risk, risk threshold; Spec, specificity; Sens, sensitivity; PPV, positive predictive value; RR, relative risk with 95% confidence interval; DLRp, positive diagnostic likelihood ratio with 95% confidence interval.

(the PPV stayed at 50%). To control the specificity of the combined test at 88% to 97%, the risk thresholds need to be lowered to 10% to 20%, giving a sensitivity of 40% to 33% (PPV 43%–72%). So for the same false-positive rate, a total of eight extra cancers (a 2.6-fold increase) were declared positive, all (in this case) with a pretest risk between 20% and 60% (Table 4 and Supplementary Table 2). So lower-risk patients are having their cancer detected. This effect was seen only at high specificity levels.

For fixed risk threshold, for example, a threshold of 30% with the MAYO model, the PPV increased from 48% to 91% when *Early*CDT-Lung was added (Fig. 3 for MAYO and Supplementary Figs. 5 and 6 for GOULD and BROCK, respectively). Virtually all false-positive results have been eliminated (25 down to 1), with approximately half of the cancers being lost (23 down to 11) (Table 5). Also shown is the percentage of subjects whose risk is above the threshold and are thus eligible for a positive combined test.

Discussion

The study showed, under strict eligibility conditions, that the addition of a positive *Early*CDT-Lung AAb test to nodule size alone significantly increased the PPV for malignancy prediction, with relative risks of 2.7-fold for nodules 4 to 20 mm in the largest diameter.

Using nodule-based risk models with positivity thresholds, lowering the risk threshold, and adding the AAb test using a both-positive rule increased sensitivity by reclassifying a proportion of the lower-risk false-negatives to true positives. This was demonstrated in 4- to 20-mm nodules, which is important because malignant nodules smaller than 20 mm are largely stage IA.[4,15] Alternatively, the false-positive rate can be reduced while still detecting a reasonable number of cancers by fixing the risk threshold for the combined test.

The testing was not controlled by a formal protocol and may have been subject to biases, particularly with respect to diagnosis not based on a pathology report, hence the two cohorts. However, the study reflects the clinical setting within which a biomarker test might be expected to operate, and so the findings do have validity. Additionally, as indicated previously,[9] those with a negative test were also followed up to avoid observer bias and allow unbiased estimates of the relative risk. The risk model analysis is, of course, only approximate. The models themselves were trained on specific data sets with different nodule size distributions and malignancy rates.[14] We hope to confirm the results in a prospective study.

Lung cancer tends to be detected at a later stage as a nodule grows.[4] A biomarker that suggests an increased probability of malignancy while the nodule is still relatively small could lead to early detection and decreased mortality. We have shown that an AAb test can significantly add to clinicians' interpretation of pulmonary nodules, both with and without risk models, for nodules smaller than 20 mm in diameter. For larger nodules (e.g., >20 mm) there is less need for a biomarker test because the clinical pathway is better established. For indeterminate nodules and a negative AAb test, the physician should continue to assess patients on the basis of their other risk factors and subsequent scans.

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Supplementary Data

Note: To access the supplementary material accompanying this article, visit the online version of the *Journal of Thoracic Oncology* at www.jto.org and at http://dx.doi.org/10.1016/j.jtho.2016.08.143.

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Tumor-Associated Autoantibodies: Re-Optimization of EarlyCDT-Lung Diagnostic Performance and Its Application to Indeterminate Pulmonary Nodules

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Abstract

Background: Low-dose computed tomography (CT) screening reduces lung cancer mortality but costs are prohibitive for most healthcare budgets due to high false positive rates. An adjunctive test able to distinguish malignant from benign pulmonary nodules would be hugely beneficial. EarlyCDT-Lung measures serum autoantibodies to tumor-associated antigens and has found clinical acceptance to aid early detection of lung cancer for high risk patients. However performance was optimized for screening. The construction of a receiver-operating characteristic (ROC) curve would enable optimization of performance for alternative settings, including nodule malignancy. Methods: A Monte-Carlo search method was used to construct a ROC curve using a case-control cohort, enabling high and low specificity versions of EarlyCDT-Lung to be determined. These were used for a theoretical evaluation of a nodule cohort, and positive predictive value (PPV) was calculated under the assumption of independence of risk source. Patients or their nodules are typically classified into three risk groups: low (0% - 10%), intermediate (10% - 65%) and high (>65%) risk of malignancy. The predicted shift in risk group by application of the high and low specificity versions, along with the current commercial EarlyCDT-Lung, was then estimated. Results: The ROC curve, with an area under the curve of 0.743, was constructed. The high specificity (98%), low specificity (49%) and current commercial (91% specificity) versions of EarlyCDT-Lung re-classified 27%, 23% and 26% of intermediate nodules, respectively, to either a higher (10%, 8% and 10%) or lower (17%, 15% and 16%) risk group. Conclusion: A ROC curve was constructed to allow performance prediction of EarlyCDT-Lung at different specificities in the indeterminate nodule setting. This enabled risk re-classification of intermediate risk nodules, and could therefore facilitate alternative more appropriate in-

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tervention. We have shown how a multivariate biomarker test can add to the interpretation of pulmonary nodules and therefore aid patient management.

Keywords

Tumor-Associated Autoantibodies, Indeterminate Pulmonary Nodules, Diagnostic Screening, Computed Tomography, ROC Curve

1. Introduction

In the United States, there is a well-documented high rate of lung cancer and pulmonary nodules [1] [2]. In recent years, widespread use of computed tomography (CT) resulting from the finding by the National Lung Screening Trial that annual CT screening reduces lung cancer mortality by 20% compared to chest x-ray [3] has resulted in over 50% of CT-screened patients having at least one non-calcified nodule and over 96% of nodules over 4mm being benign (false positive) [3] [4]. There is also a high false positive rate and morbidity associated with biopsy or resection of these benign nodules [2] [3] [5]. Consequently, a CT screening program will need substantial resources to perform follow up for every pulmonary nodule detected. Potential malignancy has been found to increase with nodule size, which also tends to result in later-stage detection of lung cancer as the nodule grows [4]. Therefore, there is a clear need for a complimentary test to aid evaluation of nodule malignancy potential, ideally a biomarker, to reduce false positive rates of CT screening for lung cancer and enable earlier detection.

Tumor-associated (TA) antigens are currently the most commonly measured biomarkers during management of cancer patients [6]. However they are often of little utility in early disease due to serum levels having a strong correlation with tumor burden. This generally results in levels only being elevated in later stage disease, limiting their clinical use to monitoring of treatment and disease recurrence. TA autoantibodies have been identified for a range of solid tumors and are currently emerging as strong candidates for clinically useful cancer biomarkers [7]. The mechanisms of their secretion have not been clearly determined but their production is thought to occur due to increased immunogenicity of the corresponding antigen. They are produced early in tumorigenesis, being measurable up to 5 years before the development of clinical symptoms [8]. As antibodies they represent biologically amplified markers, increasing the detectable signal for the corresponding antigen. They also persist in the circulation with half-lives of typically up to 30 days [9] and are more stable outside the body than other biomarkers.

EarlyCDT-Lung (Oncimmune Holdings plc.) detects the presence of TA autoantibodies to a panel of seven lung cancer associated antigens using an indirect enzyme-linked immunosorbent assay (ELISA) method [10] [11]. A sample is positive when at least one of the panel of TA autoantibodies is elevated above a

pre-determined cut-off [12]. The test has been both technically [10] and clinically validated in seven independent validation cohorts [11] [13] including high-risk control groups matched for age, sex, and smoking history. The performance characteristics have been further validated in the commercial setting by an audit of clinical outcomes for the first 1599 patients who had a valid EarlyCDT-Lung and unknown nodule status [14]. The test consistently identifies lung cancer with 92% accuracy (compared with 50% for CT) with a sensitivity (true positive rate) of ~40% for all stages and types (small cell and non-small cell) of lung tumors and a specificity (true negative rate) of ~93% for all cohorts, showing the robustness and reproducibility of this assay system [12].

The test was launched commercially in 2012 for the early detection of lung cancer in a high-risk screening scenario [14] [15]. However it has also had some clinical acceptance for follow up of patients who had a positive result on CT. It has recently been demonstrated that a positive test result reflects a significant increased risk for malignancy in lung nodules 4 to 20 mm in diameter [16]. This confirms that EarlyCDT-Lung may have clinical value in assessment of malignancy risk for indeterminate pulmonary nodules.

The optimum diagnostic performance of EarlyCDT-Lung in the screening setting may not be appropriate for the indeterminate nodule setting. Performance is defined by the particular set of cut-offs chosen. So by varying the cut-offs, specificity and sensitivity, and hence false-positive and false-negative rates, can be adjusted. So the purpose of this present paper is largely technical, and describes the creation and use of a receiver-operating characteristic (ROC) curve for EarlyCDT-Lung. Because of the need for a relatively large number of cancers to achieve this, a case-control cohort (designated the optimization cohort) previously used for validation of EarlyCDT-Lung was used [11] [12]. It was compared with a dataset (designated the nodule cohort) derived from our commercial operations (designated the audit cohort).

2. Materials and Methods

2.1. Patient Cohorts

2.1.1 Optimization Cohort

This cohort has been described elsewhere and was previously used to set the current commercial cut-offs for EarlyCDT-Lung [12]. Briefly, serum samples from 235 patients with lung cancer obtained at or just after histopathological confirmation of the tumor, were assayed. The lung cancers consisted of 178 non-small cell lung cancer (75.7 %), 53 small cell lung cancers (22.6 %), and 4 others (1 sarcoma, 2 × bronchogenic carcinomas, and 1 undefined lung cancer). This cohort was representative of a high risk population with a mean and median age of 65 years and a high proportion of smokers (49%) and ex-smokers (29%). The controls consisted of 266 healthy volunteers, 235 of which were individually matched to the lung cancer patients for age, gender, and smoking status. This group of controls had no evidence of any current or prior cancer including non-melanoma skin cancer.

2.1.2. Audit Patient Cohort

This cohort has been described previously [14]. Briefly, EarlyCDT-Lung was launched commercially in November 2010 with physicians in routine practice across USA ordering the test on behalf of their patients. This cohort was assembled for an audit of clinical practice, reporting the physicians' use of the test and not a prospective study in a population defined by inclusion and exclusion factors. The cohort is comprised of the first 861 patients tested with the seven-antigen test, with some exclusion factors applied. The cohort was followed for clinical outcomes and a total of 35 cases of lung cancer were recorded.

2.1.3. Nodule Patient Cohort

This cohort was derived from the audit cohort by assessing CT imaging reports, from within 6 months of the autoantibody test, for the presence of pulmonary nodules. The size of the largest non-calcified nodule was recorded and exclusion criteria (invalid autoantibody tests, lost-to-follow-up or with previous history of cancer) were applied. The "Exclusive cohort" [14], tested with the seven antigen panel and with cancer being confirmed by pathology reports (n=166) [16], was used. Nodules were categorized by size (<4 mm, 4 mm - 20 mm, >20 mm).

2.2. Comparison of Cohorts

The diagnostic performance of the optimization, audit and nodule (by nodule size: <4 mm, 4 mm - 20 mm and >20 mm) cohorts was compared statistically using Fisher's Exact test applied to frequency tables for specificity and sensitivity separately. The test is slightly biased in the direction of non-significance due to the nodule cohort being a subset of the audit set.

2.3. Construction of ROC Curve

When a diagnostic test is based on a single test result or score, the construction of a ROC curve is straightforward. The cut-off can be varied from minimum to maximum possible values and the specificity and sensitivity are read off at a series of points. EarlyCDT-Lung, however, is effectively a multivariate test, so construction of a ROC curve is not as straightforward. Since the cut-offs for EarlyCDT-Lung are based on quantitative results, one method is to re-optimize the test at every point along a range of selected specificity levels. For the optimization cohort individualized antigen cut-offs were re-optimized to maximize sensitivity at each and every specificity level hence allowing the construction of a complete ROC curve. The optimization was a paper exercise not involving re-assay of the samples.

A Monte-Carlo direct search was used to explore the large number of possible combinations of cut-off level as follows. For a set of n controls:

1) Choose the specificity.
2) Calculate the number of control subjects that can be specified as positive *i.e.* s = n (1-specificity).
3) Select an autoantibody at random.
4) Select at random a number (r) between 1 and s. Identify the r samples with

the highest OD for the autoantibody selected in step 3 positive, ignoring those already declared positive.

5) Set each cut-off value above the highest non-positive control sample OD value for that autoantibody.

6) Repeat steps 3 and 4 a 1000 times until the total number of control samples specified as positive is equal to the number set in step 2.

7) Calculate sensitivity.

8) Repeat the process for the next value of specificity.

9) Plot the ROC curve, i.e. sensitivity vs. (1-specificity) for all values of specificity.

To complete the description of diagnostic performance, the PPV, Negative Predictive Value (NPV) and Relative Risk (RR) were calculated based on 20% lung cancer risk for patients with nodules (of any size) [16]. Positive diagnostic ratio (DLRp) and Negative diagnostic ratio (DLRn) were also calculated.

2.4. Shift of Risk Category

Patients or their nodules are typically classified into three groups: low (0% - 10%), intermediate (10% - 65%) and high (>65%) risk of malignancy; although these ranges vary with source. The use of a biomarker test such as EarlyCDT-Lung will change a patient's pre-test risk to a post-test risk, possibly resulting in a shift of risk group. Low and high risk groups will have clear monitoring and intervention paths. Nodules with intermediate risk are not so easy to manage clinically. So we calculated what percentage of intermediate nodules are re-classified using the different versions of the EarlyCDT-Lung test expressed in the ROC curve.

We took the current commercial test (with 91% specificity and 40% sensitivity for the optimization cohort), and determined a high specificity version of the test (with 98% specificity and 28% sensitivity for the optimization cohort) and a low specificity version of the test (with 49% specificity and 80% sensitivity for the optimization cohort). First we used the Swensen/Mayo nodule-based risk model [17] [18] to derive the distribution of pre-test risk for all the nodule cases in the nodule cohort. Next we calculated the frequency of nodules in each 5% risk category. For each category, with midpoint risk r, we then calculated the predicted post-test risk *i.e.* the PPV given a positive marker test and (100-NPV) given a negative test using the usual formulae where sens is sensitivity and spec is specificity, and here with all factors expressed as proportions rather than percentages:

$$\mathrm{PPV} = \frac{\mathrm{r} \times \mathrm{sens}}{(\mathrm{r} \times \mathrm{sens}) + (1-\mathrm{r})(1-\mathrm{spec})} \tag{1}$$

$$\mathrm{NPV} = \frac{(1-\mathrm{r})\mathrm{spec}}{\mathrm{r}(1-\mathrm{sens}) + (1-\mathrm{r})\mathrm{spec}} \tag{2}$$

Next we calculated the predicted numbers (no.) of nodules positive and negative for the test:

$$\text{No. true positive} = \text{frequency of category} \times \text{predicted no. cancers} \times \text{sensitivity} \quad (3)$$

$$\text{No. false negative} = \text{frequency of category} \times \text{predicted no. cancers} \times (1 - \text{sensitivity}) \quad (4)$$

$$\text{No. true negative} = \text{frequency of category} \times \text{predicted no. controls} \times \text{specificity} \quad (5)$$

$$\text{No. false positive} = \text{frequency of category} \times \text{predicted no. controls} \times (1 - \text{specificity}) \quad (6)$$

Hence the number shifted up to a higher risk group is the total of true positives and true negatives where the post-test risk is in a higher risk group and the number shifted down to a lower risk group is the total of true negatives and false negatives where the post-test risk is in a lower risk group.

2.5. Diagnostic Likelihood Ratio (DLRp)

In a clinical setting pre-test and post-test risk estimation is generally more useful than specificity and sensitivity themselves. With a pre-test risk, r, and making the assumption of statistical independence mentioned above, the process equates to applying the positive diagnostic likelihood ratio (DLRp) [19] for test-positive subjects where:

$$\text{logit}(PPV) = \text{logit}(r) + \ln(DLRp) \left[\text{where logit}(x) \text{ defined as } \ln\left(x/(1-x)\right) \right] \quad (7)$$

There is an equivalent formula for test-negative subjects which converts the pre-test risk to post-test (NPV) using the negative ratio (DLRn) involving NPV:

$$\text{logit}(\text{NPV}) = -\text{logit}(\text{r}) - \ln(\text{DLRn}) \quad (8)$$

Expressing the conversion from pre- to post-test risk in this way emphasizes that these estimates of post-test risk rely on the assumption of strict statistical independence of nodule size and marker test under a "both-positive rule".

3. Results

3.1. Comparison of Test Performance for the Patient Cohorts

The diagnostic metrics (specificity, sensitivity and positive diagnostic likelihood ratio) were first tabulated (**Table 1**). The current commercial EarlyCDT-Lung performance for the nodule cohort (specificity of 85.6% and sensitivity of 37.8%) was well in-line with that of the optimization cohort (specificity of 90.6% and sensitivity of 41.3%) bearing in mind the confidence intervals. Performance was also maintained when assessing the nodule cohort by nodule size where for nodules of 4 - 20 mm specificity was 83.9% with sensitivity of 40.0% whereas for nodules >20 mm specificity of 91.7% and sensitivity of 36.4% were determined. There were no nodules <4 mm. The performance comparison for the cohorts did not approach significance for either specificity ($p = 0.28$) or sensitivity ($p = 0.93$), despite the varying cancer rate (2.3% to 47.8%).

The performance was also compared statistically for the cohorts by calculating DLRp (see **Table 1**) in order to assess whether these cohorts were similar enough to be used for this study. For the audit and nodule cohorts, due to the relatively low number of cancers the DLRp is not estimated with high accuracy, and

Table 1. Summary of test performance for the patient cohorts.

Cohort	Performance parameters					
	Number of cancer samples	Number of control samples	Specificity %	Sensitivity %	Cancer rate %	DLRp
Optimisation	235	266	90.6 (87.1 - 94.1)	41.3 (35.0 - 47.6)	-	4.4 (2.9 - 6.6)
Audit	19	817	90.5 (88.4 - 92.5)	47.4 (24.9 - 69.8)	2.3	5.0 (3.0 - 8.3)
Nodule[c] (4 - 20 mm)	15	87	83.9 (76.2 - 91.6)	40.0 (15.2-64.8)	14.7	2.5 (1.1 - 5.4)
Nodule[c] (>20 mm)	22	24	91.7 (80.6 - 100)	36.4 (16.3 - 56.5)	47.8	4.4 (1.0 - 18.4)
Nodule	37	111	85.6 (79.1 - 92.1)	37.8 (22.2 - 53.5)	22.3	2.6 (1.4 - 4.8)

[a]95% confidence intervals for specificity, sensitivity and DLRp are shown in brackets, [b]Comparison of first four cohorts using Fisher's Exact test: specificity p = 0.28, sensitivity p = 0.93, [c]Nodule subset, size range in brackets.

showed quite wide confidence intervals, but the estimates were consistent across cohorts.

The consistency of diagnostic performance of EarlyCDT-Lung for the nodule and audit cohorts was determined to be similar to that for the optimization cohort. Therefore the ROC curve constructed using the optimization cohort can be used to predict the performance of higher and lower specificity versions of EarlyCDT-Lung and the subsequent nodule risk category shift analysis for the nodule and audit cohorts.

3.2. ROC Curve

The performance statistics were tabulated for a range of 100 specificity values from 0% to 100% allowing the creation of the whole ROC curve (**Figure 1**). A selection of the higher specificity points, 49% to 98%, with sensitivity ranging from 80% to 28%, were also tabulated with relevant performance metrics (**Table 2**).

The estimated area under the curve (AUC) for the ROC curve was 0.743 ($p < 0.001$ vs. randomness). The PPV, relative risk, DLRp and DLRn all increase as specificity increases. Thus between 90% and 98% specificity the diagnostic performance increases considerably, PPV from 54% to 78%, relative risk from 3.7 to 5.0 and DLRp from 4.2 to 14.0. The increase for DLRn was more modest, from 0.64 to 0.73. NPV increases steadily as specificity decreases, reaching 90.7% at a specificity of 49%. These changes result from the combination of high specificity and relatively low cancer rate which results in the number of false-positives falling rapidly towards the top of the table. High PPV is of use to a clinician faced with a positive biomarker test. Higher DLRp values mean that a positive biomarker test has greater relative effect on a pre-test risk.

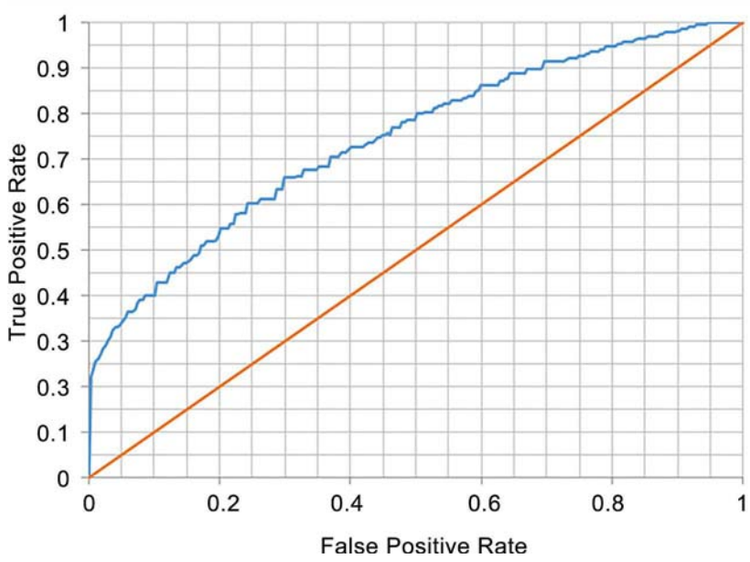

Figure 1. Maximum true positive rate ROC curve constructed from multivariate data from the optimisation cohort. AUC = 0.743 ($p < 0.001$ vs. randomness).

Table 2. Tabulated performance characteristics of EarlyCDT-Lung for a range of specificities[a].

Specificity %	Sensitivity %	PPV %	NPV %	RR	DLRp	DLRn
98	28	78	84.5	5.0	14.0	0.73
97	30	71	84.7	4.7	10.0	0.72
96	33	67	85.1	4.5	8.3	0.70
95	34	63	85.2	4.3	6.8	0.69
94	35	59	85.3	4.0	5.8	0.69
93	37	57	85.5	3.9	5.3	0.68
92	38	54	85.6	3.8	4.8	0.67
91	40	53	85.8	3.7	4.4	0.66
90	42	51	86.1	3.7	4.2	0.64
87	45	46	86.4	3.4	3.5	0.63
82	50	41	86.8	3.1	2.8	0.61
79	55	40	87.5	3.2	2.6	0.57
74	60	37	88.1	3.1	2.3	0.54
68	65	34	88.6	3.0	2.0	0.51
62	70	32	89.2	2.9	1.8	0.48
55	75	29	89.8	2.9	1.7	0.45
49	80	28	90.7	3.0	1.6	0.41

[a]Based on 20% cancer prevalence expected in a nodule cohort.

3.3. Shift of Risk Category

The risk groups and performance statistics for three test versions of interest, high specificity (98% specificity and 28% sensitivity for the optimization cohort),

current commercial test (91%/40%) and low specificity (49%/80%) were tabulated (**Table 3**) with performance metrics. Any nodule category which had switched risk group was highlighted. For the high specificity version a total of 26.9% of intermediate nodules were re-classified either to a higher (10.1%) or lower (16.8%) risk group, whilst for the current commercial test a total of 25.9% were re-classified, either to a higher (10.4%) or lower (15.5%) group, and for the low specificity version of the test a total of 22.5% were re-classified, either to a higher (7.6%) or lower (14.9%) group.

4. Discussion

The current commercial EarlyCDT-Lung performance in a pre-imaging screening scenario is quoted at 90.6% specificity and 41.3% sensitivity, with the speci-

Table 3. EarlyCDT-Lung performance characteristics of the nodule cohort by risk category with predicted category shift.

		High specificity version (98%/28%)[c]			Current commercial test (91%/40%)[c]			Low specificity version (80%/49%)[c]		
Risk[a] %	Frequency[b] %	PPV[d] %	100-NPV[e] %	RR[f]	PPV[d] %	100-NPV[e] %	RR[f]	PPV[d] %	100-NPV[e] %	RR[f]
LOW										
0 - 5	15	26.4	1.8	14.3	10.2	1.7	6.2	3.9	1.0	3.7
5 - 10	24	53.2	5.6	9.5	26.5	5.1	5.2	11.3	3.2	3.5
MID										
10 - 15	8	66.7	9.5	7.0	38.8	8.6	4.5	18.3	5.5	3.3
15 - 20	7	74.8	13.5	5.5	48.5	12.3	4.0	25.0	8.0	3.1
20 - 25	6	80.3	17.6	4.6	56.3	16.1	3.5	31.3	10.6	3.0
25 - 30	4	84.2	21.8	3.9	62.8	20.0	3.1	37.3	13.4	2.8
30 - 35	3	87.1	26.1	3.3	68.2	24.1	2.8	43.0	16.4	2.6
35 - 40	3	89.4	30.6	2.9	72.7	28.3	2.6	48.5	19.7	2.5
40 - 45	3	91.2	35.2	2.6	76.7	32.8	2.3	53.7	23.2	2.3
45 - 50	3	92.7	39.9	2.3	80.1	37.4	2.1	58.7	27.0	2.2
50 - 55	3	93.9	44.8	2.1	83.1	42.2	2.0	63.4	31.1	2.0
55 - 60	3	95.0	49.8	1.9	85.7	47.1	1.8	68.0	35.6	1.9
60 - 65	2	95.9	55.0	1.7	88.1	52.4	1.7	72.3	40.5	1.8
HIGH										
65 - 70	2	96.7	60.4	1.6	90.2	57.8	1.6	76.5	45.9	1.7
70 - 75	2	97.4	66.0	1.5	92.1	63.5	1.5	80.5	51.8	1.6
75 - 80	2	98.0	71.7	1.4	93.9	69.4	1.4	84.4	58.4	1.4
80 - 85	2	98.5	77.6	1.3	95.4	75.7	1.3	88.1	65.8	1.3
85 - 90	2	99.0	83.7	1.2	96.9	82.2	1.2	91.7	74.1	1.2
90 - 95	2	99.4	90.1	1.1	98.2	89.0	1.1	95.1	83.4	1.1
95 - 100	4	99.8	96.6	1.0	99.4	96.3	1.0	98.4	94.1	1.0
% Up		10.1			10.4			7.6		
% Down			16.8			15.5			14.9	

[a]pre-test risk based on Swensen/Mayo nodule-based risk model with the lower figure being inclusive *i.e.* 0.00 and the higher figure being exclusive *i.e.* 4.99, [b]frequency of nodule risk in the nodule cohort, [c](specificity/sensitivity) calculated for the optimization cohort, [d]post-test risk given a positive test result, [e]post-test risk given a negative test result, [f]relative risk = PPV/(100-NPV). Colors indicate risk groups of low (green), mid (orange) and high (red). The first column shows pre-test risk grouping, color coding for the rest of the table indicates shifted post-test risk group only for the groups that shifted.

ficity unadjusted for occult cancer in the optimization cohort [11] [12]. The performance was determined by fixing the specificity and then optimizing sensitivity. The specificity of around 90% was chosen to restrict the false-positive rate to 10%. This rate is quite high but is justified on the basis that follow-up of positive patients will be by imaging and not anything more invasive. In the situation where a patient has already undergone imaging and a nodule has been observed, then the next stage may be more invasive possibly involving biopsy or surgery. Here the false-positive level needs to be kept low to avoid unnecessary invasive procedures. So the specificity would be set at a higher level, perhaps 95% or higher.

The diagnostic performance of EarlyCDT-Lung in the nodule cohort was found similar to that for the optimization cohort and hence the ROC curve constructed (using the optimization cohort) can be used to predict the performance of higher and lower specificity versions of EarlyCDT-Lung in the nodule context. Note that the ROC curve presented here is effectively a non-parametric curve as opposed to a model-based curve derived, for example, using logistic regression. This allows multivariate optimization without the assumptions required for a model-based method.

Clinicians consider many other factors besides nodule size itself when interpreting indeterminate pulmonary nodules. In some specialist centers nodule-based risk models, incorporating both demographic and nodule characteristics, are used [18]. We combined the EarlyCDT-Lung biomarker test with a nodule-based risk estimator (Swensen/Mayo in this case) to predict the percentage of intermediate risk pulmonary nodules which switched risk group after a positive or negative biomarker test. For the high specificity, commercial and low specificity tests the nodule reclassification was as high as 27%, 26% and 23% respectively, with the majority of reclassification being in the direction of lower risk for all tests.

It is recommended for nodules of moderate size that clinicians estimate the pre-test probability of malignancy either qualitatively by using their clinical judgment or quantitatively by using a validated model. The Fleischner Society guidelines describe the recommended follow-up for patients identified with a pulmonary nodule by CT [20] [21] and balance early cancer detection and treatment against over-use of invasive diagnostic procedures. A biomarker that suggested an increased or decreased risk of malignancy whilst the nodule was still relatively small (e.g. 4 mm - 20 mm) would be clinically useful, as it could lead to earlier detection and decreased mortality. For larger nodules there is less need for a biomarker test since the clinical pathway is better established.

5. Conclusion

We have shown that it is possible to derive a receiver-operator characteristic (ROC) curve for a multivariate assay by re-optimizing the cut-offs for every fixed specificity value. This allows the deployment of different versions of the EarlyCDT-Lung biomarker test in different clinical situations depending on the relative costs of false-positives and false-negatives. In particular, higher and

lower specificity versions would seem to be more appropriate for a nodule interpretation scenario where subsequent intervention may well be invasive.

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CHEST Supplement

DIAGNOSIS AND MANAGEMENT OF LUNG CANCER, 3RD ED: ACCP GUIDELINES

Evaluation of Individuals With Pulmonary Nodules: When Is It Lung Cancer?

Diagnosis and Management of Lung Cancer, 3rd ed: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines

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Objectives: **The objective of this article is to update previous evidence-based recommendations for evaluation and management of individuals with solid pulmonary nodules and to generate new recommendations for those with nonsolid nodules.**

Methods: **We updated prior literature reviews, synthesized evidence, and formulated recommendations by using the methods described in the "Methodology for Development of Guidelines for Lung Cancer" in the American College of Chest Physicians Lung Cancer Guidelines, 3rd ed.**

Results: **We formulated recommendations for evaluating solid pulmonary nodules that measure ≥ 8 mm in diameter, solid nodules that measure <8 mm in diameter, and subsolid nodules. The recommendations stress the value of assessing the probability of malignancy, the utility of imaging tests, the need to weigh the benefits and harms of different management strategies (nonsurgical biopsy, surgical resection, and surveillance with chest CT imaging), and the importance of elicit- ing patient preferences.**

Conclusions: **Individuals with pulmonary nodules should be evaluated and managed by estimating the probability of malignancy, performing imaging tests to better characterize the lesions, evaluating the risks associated with various management alternatives, and eliciting their preferences for management.** ***CHEST 2013; 143(5)(Suppl):e93S–e120S***

Abbreviations: AAH = atypical adenomatous hyperplasia; ACCP = American College of Chest Physicians; AIS = adenocarcinoma in situ; EBUS = endobronchial ultrasound; ENB = electromagnetic navigation bronchoscopy; FDG = fluorodeoxyglucose; HU = Hounsfieldunit; LR = likelihoodratio; SPECT = single-photonemission CT; TBB = transbronchial biopsy; TTNB = transthoracic needle biopsy; VATS = video-assisted thoracic surgery; VBN = virtual bronchoscopy navigation; VDT = volume doubling time

Summary of Recommendations

General Approach

2.3.1. In the individual with an indeterminate nodule that is visible on chest radiography and/or chest CT, we recommend that prior imaging tests should be reviewed (Grade 1C).

2.3.2. In the individual with a solid, indeterminate nodule that has been stable for at least 2 years, we suggest that no additional diagnostic evaluation need be performed (Grade 2C).

Remark: This recommendation applies only to solid nodules. For guidance about follow-up of subsolid nodules, see Recommendations 6.5.1 to 6.5.4.

2.3.3. In the individual with an indeterminate nodule that is identified by chest radiography, we recommend that CT of the chest should be performed (preferably with thin sections

through the nodule) to help characterize the nodule (Grade 1C).

Solid Nodules ≤ 8 mm

4.1.1.1. In the individual with a solid, indeterminate nodule that measures ≤ 8 mm in diameter, we suggest that clinicians estimate the pretest probability of malignancy either qualitatively by using their clinical judgment and/or quantitatively by using a validated model (Grade 2C).

4.2.4.1. In the individual with a solid, indeterminate nodule that measures ≤ 8 mm in diameter and low to moderate pretest probability of malignancy (5%-65%), we suggest that functional imaging, preferably with PET, should be performed to characterize the nodule (Grade 2C).

4.2.4.2. In the individual with a solid, indeterminate nodule that measures ≤ 8 mm in diameter and a high pretest probability of malignancy (≥ 65%), we suggest that functional imaging should not be performed to characterize the nodule (Grade 2C).

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Remark: PET may be indicated for pretreatment staging among those patients with nodules in whom malignancy is strongly suspected or confirmed.

4.4.1.1. In the individual with a solid, indeterminate nodule that measures ≤ 8 mm in diameter, we recommend that clinicians discuss the risks and benefits of alternative management strategies and elicit patient preferences for management (Grade 1C).

4.5.1.1. In the individual with a solid, indeterminate nodule that measures ≤ 8 mm in diameter, we suggest surveillance with serial CT scans in the following circumstances (Grade 2C):

- **When the clinical probability of malignancy is very low (≤ 5%)**
- **When clinical probability is low (≤ 30% to 40%) and the results of a functional imaging test are negative (ie, the lesion is not hypermetabolic by PET or does not enhance ≥ 15 Hounsfield units on dynamic contrast CT), resulting in a very-low posttest probability of malignancy**
- **When needle biopsy is nondiagnostic and the lesion is not hypermetabolic by PET**
- **When a fully informed patient prefers this nonaggressive management approach.**

Remark: CT surveillance of solid nodules > 8 mm should use low-dose, noncontrast techniques.

4.5.1.2. In the individual with a solid, indeterminate nodule that measures ≤ 8 mm in diameter who undergoes surveillance, we suggest that serial CT scans should be performed at 3 to 6, 9 to 12, and 18 to 24 months, using thin sections and noncontrast, low-dose techniques (Grade 2C).

Remark: Serial CT scans should be compared with all available prior studies, especially the initial (index) CT scan.

Remark: Where available, manual and/or computer-assisted measurements of area, volume, and/or mass may facilitate early detection of growth.

4.5.1.3. In the individual with a solid, indeterminate nodule that shows clear evidence of malignant growth on serial imaging, we recommend nonsurgical biopsy and/or surgical resection unless specifically contraindicated (Grade 1C).

Remark: Solid nodules that decrease in size but do not disappear completely should be followed to resolution or lack of growth over 2 years.

4.6.2.1.1. In the individual with a solid, indeterminate nodule that measures ≥ 8 mm in diameter, we suggest nonsurgical biopsy in the following circumstances (Grade 2C)**:**

- **When clinical pretest probability and findings on imaging tests are discordant**
- **When the probability of malignancy is low to moderate (~ 10% to 60%)**
- **When a benign diagnosis requiring specific medical treatment is suspected**
- **When a fully informed patient desires proof of a malignant diagnosis prior to surgery, especially when the risk of surgical complications is high.**

Remark: The type of biopsy should be selected based on nodule size, location, and relation to a patent airway; the risk of complications in the individual patient; and available expertise.

4.6.3.1.1. In the individual with a solid, indeterminate nodule that measures ≥ 8 mm in diameter, we suggest surgical diagnosis in the following circumstances (Grade 2C)**:**

- **When the clinical probability of malignancy is high (≥ 65%)**
- **When the nodule is intensely hypermetabolic by PET or markedly positive by another functional imaging test**
- **When nonsurgical biopsy is suspicious for malignancy**
- **When a fully informed patient prefers undergoing a definitive diagnostic procedure.**

4.6.3.1.2. In the individual with a solid, indeterminate nodule measuring ≥ 8 mm in diameter who chooses surgical diagnosis, we recommend thoracoscopy to obtain a diagnostic wedge resection (Grade 1C)**.**

Remark: Use of advanced localization techniques or open thoracotomy may be necessary when resecting small or deep nodules.

Solid Nodules < 8 mm

5.3.1. In the individual with a solid nodule that measures <8 mm in diameter and no risk factors for lung cancer, we suggest that the frequency and duration of CT surveillance be chosen according to the size of the nodule (Grade 2C)**:**

- **Nodules measuring <4 mm in diameter need not be followed, but the patient should be informed about the potential benefits and harms of this approach**
- **Nodules measuring ≥ 4 mm to 6 mm should be reevaluated at 12 months without the need for additional follow-up if unchanged**
- **Nodules measuring ≥ 6 mm to 8 mm should be followed sometime between 6 and 12 months, and then again at between 18 and 24 months if unchanged.**

Remark: For the individual with multiple small, solid nodules, the frequency and duration of follow-up should be based on the size of the largest nodule.

Remark: CT surveillance of solid nodules <8 mm should use low-dose, noncontrast techniques.

5.3.2. In the individual with a solid nodule that measures < 8 mm in diameter who has one or more risk factors for lung cancer, we suggest that the frequency and duration of CT surveillance be chosen according to the size of the nodule (Grade 2C)**:**

- **Nodules measuring < 4 mm in diameter should be reevaluated at 12 months without the need for additional follow-up if unchanged**
- **Nodules measuring ≥ 4 mm to 6 mm should be followed sometime between 6 and 12 months and then again between 18 and 24 months if unchanged**
- **Nodules measuring ≥ 6 mm to 8 mm should be followed initially sometime between 3 and 6 months, then subsequently between 9 and 12 months, and again at 24 months if unchanged.**

Remark: For the individual with multiple small, solid nodules, the frequency and duration of follow-up should be based on the size of the largest nodule.

Remark: CT surveillance of solid nodules < 8 mm should use low-dose, noncontrast techniques.

Nonsolid (Pure Ground Glass) Nodules

6.5.1. In the individual with a nonsolid (pure ground glass) nodule measuring <5 mm in diameter, we suggest no further evaluation (Grade 2C)**.**

6.5.2. In the individual with a nonsolid (pure ground glass) nodule measuring ≥ 5 mm in diameter, we suggest annual surveillance with chest CT for at least 3 years (Grade 2C)**.**

Remark: CT surveillance of nonsolid nodules should use noncontrast techniques with thin sections through the nodule of interest.

Remark: Nonsolid nodules that grow or develop a solid component are often malignant, prompting further evaluation and/or consideration of resection.

Remark: Early follow-up at 3 months may be indicated for nonsolid nodules measuring ≥ 10 mm (followed by nonsurgical biopsy and/or surgical resection for nodules that persist).

Remark: Limited duration or no follow-up may be preferred by individuals with life-limiting comorbidities in whom a low-grade malignancy would be of little consequence or by others who place a high value on avoiding treatment of possibly indolent lung cancer.

Part-Solid (≥ 50% Ground Glass) Nodules

6.5.3. In the individual with a part-solid nodule measuring < 8 mm in diameter, we suggest CT surveillance at approximately 3, 12, and 24 months, followed by annual CT surveillance for an additional 1 to 3 years (Grade 2C)**.**

Remark: CT surveillance of part-solid nodules should use noncontrast techniques with thin sections through the nodule of interest.

Remark: Part-solid nodules that grow or develop a solid component are often malignant, prompting further evaluation and/or consideration of resection.

Remark: Limited duration or no follow-up may be preferred by individuals with life-limiting comorbidities in whom a low-grade malignancy would be of little consequence or by others who place a high value on avoiding treatment of possibly indolent lung cancer.

6.5.4. In the individual with a part-solid nodule measuring ≥ 8 mm in diameter, we suggest repeat chest CT at 3 months followed by further evaluation with PET, nonsurgical biopsy, and/or surgical resection for nodules that persist (Grade 2C)**.**

Remark: PET should not be used to characterize part-solid lesions in which the solid component measures < 8 mm.

Remark: Nonsurgical biopsy can be used to establish the diagnosis and/or be combined with wire, radioactive seed, or dye localization to facilitate subsequent resection. A nondiagnostic biopsy result does not exclude the possibility of malignancy.

Remark: Part-solid nodules measuring ≥ 15 mm in diameter should proceed directly to further evaluation with PET, nonsurgical biopsy, and/or surgical resection.

One or More Additional Nodules Detected During Nodule Evaluation

7.1.1. In the individual with a dominant nodule and one or more additional small nodules, we suggest that each nodule be evaluated individually and curative treatment not be denied unless there is histopathological confirmation of metastasis (Grade 2C)**.**

Remark: The classification and appropriate treatment of patients with more than one pulmonary focus of lung cancer is difficult and requires multidisciplinary consideration.

Pulmonary nodules are small, focal, rounded radiographic opacities that may be solitary or multiple. By definition, the solitary pulmonary nodule is a single, well-circumscribed, radiographic opacity that measures up to 3 cm in diameter and is surrounded completely by aerated lung.[2,3] There is no associated atelectasis, hilar enlargement, or pleural effusion. Individuals with solitary nodules are typically asymptomatic. Focal pulmonary lesions that are ≥ 3 cm in diameter are called lung masses and are presumed to represent bronchogenic carcinoma until proven otherwise. The management of individuals who present with lung masses and symptomatic nodules are discussed elsewhere.[4]

We exclude from consideration individuals with diffuse or multiple nodules, arbitrarily defined as those with ≥ 10 nodules. Diffuse nodules are more likely to be accompanied by symptoms and caused by either metastasis from extrathoracic malignancies or active infection or inflammation. Because they rarely represent bronchogenic carcinoma, they will not be discussed further. However, we include abnormalities that appear as a single dominant nodule accompanied by one or more smaller, incidental nodules, which are increasingly common and may represent the new normal given that they may be present in >50% of patients having thin-section chest CT scans. We use the term "dominant" to refer to a nodule that manifests in this pattern.

We further distinguish small, subcentimeter nodules from larger nodules because nodules that measure <8 mm in diameter are much less likely to be malignant, typically defy accurate characterization by imaging tests, and often are difficult to approach by nonsurgical biopsy. We also distinguish solid nodules from subsolid nodules. Subsolid nodules are further categorized as pure ground glass or part solid in attenuation.

Throughout this article, we use the term "indeterminate" to describe a nodule that is not calcified in a benign pattern or that does not have other features

strongly suggestive of a benign etiology, such as intranodular fat that is pathognomonic of hamartoma or a feeding artery and vein typical for arteriovenous malformation. Our recommendations apply only to indeterminate nodules. We do not distinguish screening-detected nodules from nodules that are detected incidentally, nor do we distinguish nodules that are detected by chest radiography vs chest CT scan. When evaluating individuals with lung nodules, it is more important to consider the size and morphology of the lesions as well as risk factors for malignancy and suitability for curative treatment.

We begin by updating recommendations from the second edition of these guidelines for individuals with solid nodules measuring . 8 mm in diameter (including both solitary and dominant nodules). Next, we update recommendations for evaluating solid nodules measuring <8 mm. Finally, we present a new set of recommendations for individuals with subsolid nodules.

Most of the evidence described in this article comes from uncontrolled studies of diagnostic accuracy. Although many were methodologically rigorous, all were limited by the use of accuracy as a surrogate outcome. Few randomized controlled trials or studies of higher-level outcomes have been performed. As a result, most of the recommendations are based on evidence that is relatively low in quality.[5]

1.0 Methods

To update previously published guidelines for evaluation of individuals with pulmonary nodules,[6] we repeated prior searches of MEDLINE for studies of chest CT imaging, PET imaging, and transthoracic needle biopsy (TTNB) and performed new searches for studies of subsolid nodules, bronchoscopy, surgical complications, and methods to detect nodule growth (Appendix S1). All searches were performed in October 2011 and subsequently updated through May 2012. We identified additional articles by searching our own personal files and by reviewing reference lists of included studies. We included all randomized controlled trials, controlled observational studies, uncontrolled studies of diagnostic accuracy, and cross-sectional studies that examined relationships between nodule morphology and outcomes. A multidisciplinary writing committee comprising four pulmonologists, two thoracic surgeons, and one radiologist formulated questions (Table S1), synthesized and reviewed available evidence, developed or revised recommendations, rated the quality of evidence, and graded the strength of the recommendations by using a standardized approach, as described by Lewis et al[1] in "Methodology for Development of Guidelines for Lung Cancer" in the American College of Chest Physicians (ACCP) Lung Cancer Guidelines. The writing committee reviewed all recommendations and reached consensus by iterative discussion and debate. The manuscript was extensively revised, although some sections of text (including much of the section on solid nodules measuring <8 mm in diameter) were considered to be current and, therefore, retained from the previous version. The resulting guideline supersedes the previous version. The guideline was reviewed by all members of the ACCP Lung Cancer Guidelines Panel prior to approval by the Thoracic Oncology NetWork, the Guidelines Oversight Committee, and the Board of Regents of the ACCP.

2.0 Anatomic Imaging

Pulmonary nodule diagnosis begins with imaging studies. Recent attention has focused on studies of computer-assisted detection, computer-assisted diagnosis, volumetric measurement of growth, and functional imaging, as described in this section.

2.1 Chest Radiography

Although most nodules are now detected by CT scan, many are still detected incidentally on chest radiographs that were ordered for some other purpose. Our updated literature review identified 18 potentially relevant studies of chest radiography published since 2005, including six studies of dual-energy techniques, nine studies of computer-assisted detection, and four studies of other methods to improve nodule detection (Table S2). However, none of the studies examined whether specific chest radiographic features were helpful in characterizing nodules as malignant or benign.

Occasionally, a presumptive benign diagnosis can be established when a characteristic pattern of calcification is noted on the chest radiograph. Diffuse, central, laminated, and popcorn patterns of calcification are considered to be benign.[7,8] Although often missing, the presence of intranodular fat density and popcorn calcification are specific for hamartoma.[9] If one of these patterns of calcification is clearly evident on the chest radiograph, no additional evaluation is necessary. However, other patterns of calcification, including the stippled and eccentric patterns, do not exclude malignancy. Further evaluation of these nodules is considered mandatory.

2.2 Chest CT Scan

As is true for nodules identified by chest radiography, all previous CT scans should be reviewed when a nodule is first identified by CT scan. Chest CT scan also provides specific information about the location, shape, margins, and attenuation characteristics of nodules. In addition, CT scan sometimes identifies unsuspected lymphadenopathy, synchronous parenchymal lesions, or invasion of the chest wall or mediastinum. Selected morphologic characteristics are described next. We discuss nodule size and attenuation characteristics (solid vs subsolid) in greater detail in a subsequent section.

Morphologic characteristics on chest CT scan that suggest malignancy include spiculated margins[10-12]; vascular convergence (which suggests vascular and

lymphatic invasion)[13]; and either a dilated bronchus leading into the nodule[14] or the presence of pseudocavitation, which has a bubbly appearance believed to represent air bronchiolograms.[12] True cavitation, especially when associated with a thick and irregular wall, is a strong predictor of malignancy.[15]

Our search identified seven recent studies of CT image characteristics (summarized in Appendix S2). One such study of 213 patients with nodules (92% solid) from Denmark confirmed many of the distinguishing characteristics first reported by Siegelman et al[10] and Zerhouni et al.[11] In the Danish study, a malignant (vs benign) diagnosis was more than five times more likely for nodules with spiculated or ragged margins (likelihood ratio [LR], 5.5), almost twice as likely when pleural retraction was present (LR, 1.9), and 70% more likely when a vessel sign was present (LR, 1.7) but only 10% more likely when margins were lobulated (LR, 1.1).[16] Malignancy was 30% less likely when a bronchus sign was present (LR, 0.7) and five times less likely for smooth or polygonal margins (LR, 0.2). Noncalcified nodules were equally likely to be malignant or benign (LR, 1.0). Qualitative assessment and subjective weighting of these features yielded a sensitivity of 98% for identifying malignancy but a specificity of only 23%.

Data from the NELSON (Dutch Belgian Randomised Lung Cancer Screening) trial of CT scan screening showed that for solid nodules, malignancy was associated with larger nodule size, spiculated margins, and irregular shape but not with attenuation characteristics.[17] In this study, the combination of round shape, smooth margins, and low attenuation (solid nodule with a negative CT scan number) was 100% predictive of benignity. Two other screening studies reported conflicting results about potential predictors of resolving (and therefore benign) nodules.[18,19]

Other studies have used computer-assisted techniques or novel CT scan parameters to discriminate between malignant and benign nodules, but these have been limited by small size, imprecision, and lack of external validation.[20,21] Consequently, although CT scan morphology often helps to estimate the probability of malignancy, it is rarely conclusive.

Risks associated with CT scan include radiation exposure and adverse effects because of administration of iodinated contrast material. The risk of radiation-induced cancer is uncertain in magnitude. Most studies used models that are based on nonmedical sources of ionizing radiation and concluded that risks were small but nontrivial at the population level.[22,23] Although controversy exists, less radiation exposure is obviously preferable, and the use of radiation-limiting innovations, including dose modulation and iterative reconstruction techniques, should be used when available to minimize the risks associated with repeated exposures.[24] IV contrast is relatively or absolutely contraindicated in patients with renal insufficiency or allergy to iodine, and it is usually not necessary to administer contrast when performing follow-up CT scans to identify growth.

2.3 Recommendations

2.3.1. In the individual with an indeterminate nodule that is visible on chest radiography and/or chest CT, we recommend that prior imaging tests should be reviewed (Grade 1C)**.**

2.3.2. In the individual with a solid, indeterminate nodule that has been stable for at least 2 years, we suggest that no additional diagnostic evaluation need be performed (Grade 2C)**.**

Remark: This recommendation applies only to solid nodules. For guidance about follow-up of subsolid nodules, see recommendations 6.5.1 to 6.5.4.

2.3.3. In the individual with an indeterminate nodule that is identified by chest radiography, we recommend that CT of the chest should be performed (preferably with thin sections through the nodule) to help characterize the nodule (Grade 1C)**.**

3.0 Suitability for Surgery or Other Curative-Intent Treatment

Before embarking on a potentially inconvenient, risky, and expensive evaluation, it is important to establish the individual's suitability and desire for curative treatment. Although therapeutic lobectomy frequently is contraindicated in individuals with advanced comorbid conditions, relatively few individuals will be excluded from consideration for sublobar resection or other less-invasive treatments (see Brunelli et al[25] "Physiologic Evaluation of the Patient With Lung Cancer Being Considered for Resectional Surgery" in the ACCP Lung Cancer Guidelines). However, some individuals may prefer no treatment, particularly those with life-limiting comorbid conditions. In such individuals, it does not make sense to pursue biopsy or aggressive CT scan surveillance, although it is always prudent to monitor for symptoms that may be palliated.

For individuals who desire treatment but either refuse or cannot tolerate surgery (even limited resection), surgical diagnosis is precluded. Other options for evaluation include functional imaging, CT scan surveillance, and nonsurgical biopsy. Prior to beginning nonsurgical treatment, the diagnosis of lung cancer ideally should be confirmed by biopsy specimen.

Alternatives to surgical treatment include external beam radiation, stereotactic radiotherapy, and radiofrequency ablation. Except for the section on surgical diagnosis (which applies only to surgical candidates), the remainder of this guideline applies to individuals who are candidates for curative treatment with either surgery or one of these other alternatives.

4.0 Solid Nodules Measuring ≥ 8 mm in Diameter

Among individuals with a solid nodule measuring ≥ 8 mm in diameter (either solitary or dominant), steps in the evaluation include estimating the probability of cancer; further characterizing the lesion with CT scan, PET scan, or another functional imaging test; and choosing among nonsurgical biopsy, surgical resection, and active surveillance with serial CT scans (Figs 1, 2).

4.1 Clinical Probability of Cancer

Although clinical and radiographic characteristics cannot reliably distinguish between benign and malignant nodules in most individuals, it is nevertheless important to estimate the clinical probability of malignancy before ordering imaging tests or biopsy procedures (Fig 3). Estimating pretest probability facilitates the selection and interpretation of subsequent diagnostic tests. Common sense argues that different management approaches are called for in a 30-year-old nonsmoker with an 8-mm smooth-bordered nodule and a 70-year-old heavy smoker with a 2.5-cm spiculated nodule. Most individuals with nodules have characteristics that fall somewhere between these two extremes.

Although many clinicians estimate pretest probability intuitively, several quantitative models have been developed to assist in this task,[26,30,31] including four new models developed since 2005 (Tables S3, S4).[27-29,32]

Figure 1. [Sections 4.0, 4.3] Management algorithm for individuals with solid nodules measuring 8 to 30 mm in diameter. Branches indicate steps in the algorithm following nonsurgical biopsy. *Among individuals at high risk for surgical complications, we recommend either CT scan surveillance (when the clinical probability of malignancy is low to moderate) or nonsurgical biopsy (when the clinical probability of malignancy is moderate to high). RFA = radiofrequency ablation; SBRT = stereotactic body radiotherapy.

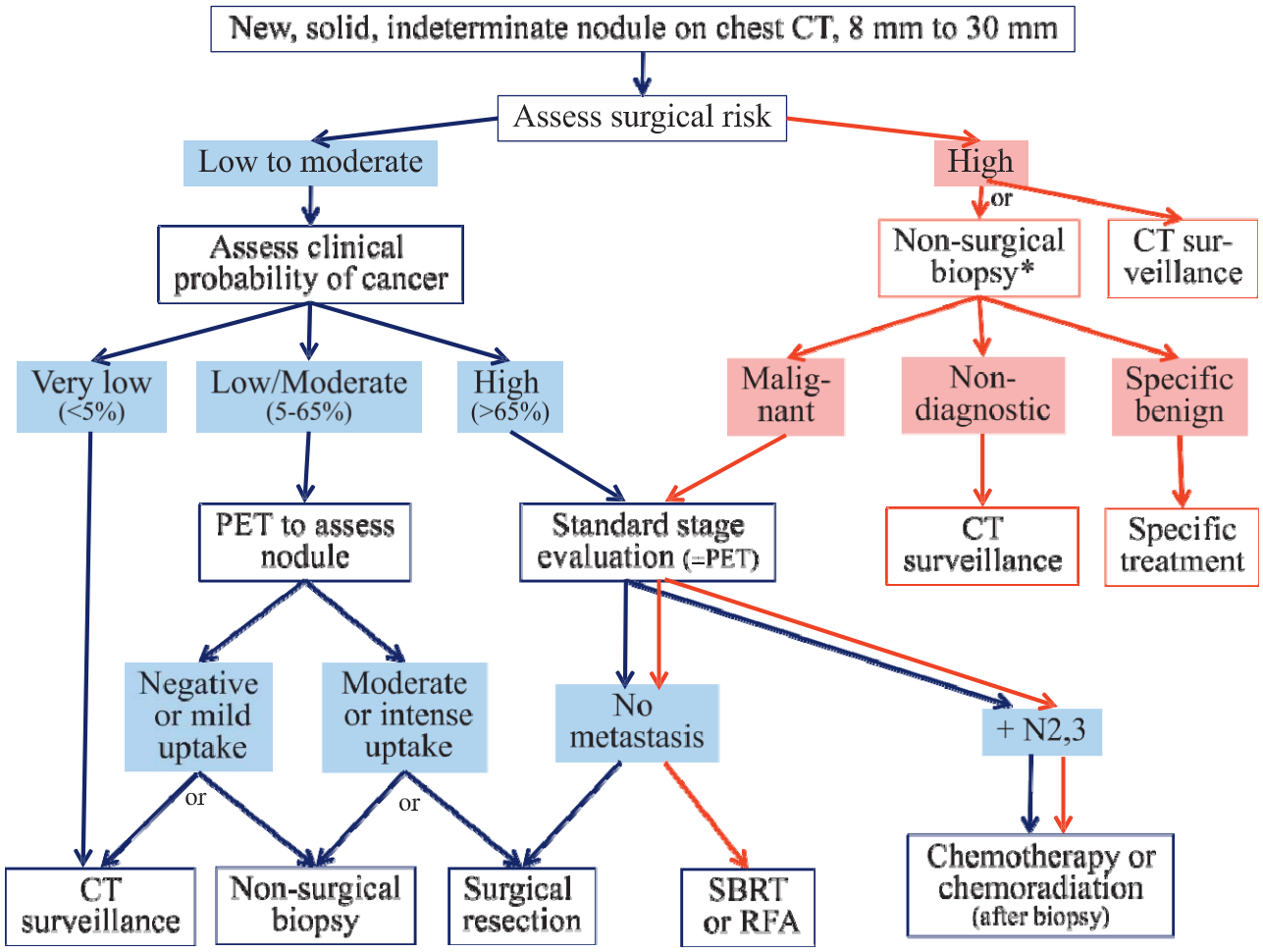


Figure 2. [Section 4.0] Factors that influence choice between evaluation and management alternatives for indeterminate, solid nodules > 8 to 30 mm in diameter.

Factor	Level	CT Scan Surveillance	PET Imaging	Nonsurgical Biopsy	VATS Wedge Resection
Clinical probability of lung cancer	Very low (< 5%)	++++	-	-	-
	Low-moderate	+	+++	−−	+
	High (< 65%)	-	(± staging)	++	++−−
Surgical risk	Low	++	++	−−	+++
	High	++	+++	−−	-
Biopsy risk	Low	-	++	−−−	+++
	High	+ +	+ + +	-	+
High suspicion of active infection or inflammation		-	-	+−−−	++
Values and preferences	Desires certainty	-	+	· · ·	+ + + +
	Risk averse to procedure-related complications	+++−	+++	−−	-
Poor adherence with follow-up		-	-	+−−	++++

VATS = video-assisted thoracoscopic surgery.

Three models have undergone external validation since 2005 (Tables S5, S6).[33-35] Another used data from the PLCO (Prostate, Lung, Colorectal, Ovarian Cancer Screening Trial) of lung cancer screening with chest radiography and found that although a lung mass (not surprisingly) was highly predictive of malignancy (OR, 11.2; 95% CI, 6.3-19.9), the finding of a lung nodule was not (OR, 1.4; 95% CI, 0.8-2.5).[36]

The most extensively validated model was developed by investigators at the Mayo Clinic who used multiple logistic regression analysis to identify six independent predictors of malignancy in 419 patients with noncalcified nodules that measured between 4 and 30 mm in diameter on chest radiography.[26,33] Independent predictors of malignancy included older age (OR, 1.04 for each year), current or past smoking history (OR, 2.2), history of extrathoracic cancer . 5 years before nodule detection (OR, 3.8), nodule diameter (OR, 1.14 for each millimeter), spiculation (OR, 2.8), and upper lobe location (OR, 2.2). The prediction model is described by the following equations:

$$\text{Probability of malignancy} = e^x / (1 + e^x) \quad \text{(Equation 1)}$$

$$x = -6.8272 + (0.0391 \times \text{age}) + (0.7917 \times \text{smoke}) + (1.3388 \times \text{cancer}) + (0.1274 \times \text{diameter}) + (1.0407 \times \text{spiculation}) + (0.7838 \times \text{location}) \quad \text{(Equation 2)}$$

Figure 3. [Section 4.1] Assessment of the probability of malignancy.

Assessment Criteria	Probability of Malignancy		
	Low (< 5%)	Intermediate (5%- 65%)	High (> 65%)
Clinical factors alone (determined by clinical judgment and/or use of validated model)[a]	Young, less smoking, no prior cancer, smaller nodule size, regular margins, and/or non-upper-lobe location		
FDG-PET scan results	Low-moderate clinical probability and low FDG-PET activity	Weak or moderate FDG-PET scan activity	Intensely hypermetabolic nodule
Nonsurgical biopsy results (bronchoscopy or TTNA)	Specific benign diagnosis	Nondiagnostic	Suspicious for malignancy
CT scan surveillance	Resolution or near-complete resolution, progressive or persistent decrease in size,[b] or no growth over ≥2 y (solid nodule) or ≥ 3-5 y (subsolid nodule)	NA	Clear evidence of growth

FDG = fluorodeoxyglucose; NA = not applicable; TTNA = transthoracic needle aspiration.
[a]In three studies, independent risk factors for malignancy included older age, current or former smoking, history of extrathoracic cancer . 5 y prior to nodule detection, larger nodule diameter, spiculated margins, and upper-lobe location[26]; older age, current or former smoking, shorter time since quitting smoking, and larger nodule diameter[27]; and high serum C-reactive protein level, high serum carcinoembryonic antigen level, absence of calcification, spiculation, and CT scan bronchus sign.[28] In another study, the combination of smooth or lobulated borders, irregular shape, and solid attenuation had a negative predictive value of 86%.[29]
[b]Approximately 20% of observed cancers have decreased in size at least at some point during the observation period.

where e is the base of natural logarithms, age is the patient's age in years, smoke51 if the patient is a current or former smoker (otherwise50), cancer51 if the patient has a history of an extrathoracic cancer that was diagnosed .5 years ago (otherwise50), diameter is the diameter of the nodule in millimeters, spiculation51 if the edge of the nodule has spicules (otherwise50), and location51 if the nodule is located in an upper lobe (otherwise50).

Of note, the accuracy of models for predicting malignancy appears to be similar to that of expert clinicians, although the correlation between models and experts is poor, suggesting that the models may provide unique information.[37,38] The choice of model might best be guided by the characteristics of the target population, ease of use, and the extent of validation.

4.1.1 Recommendation

4.1.1.1. In the individual with a solid, indeterminate nodule that measures ~ 8 mm in diameter, we suggest that clinicians estimate the pretest probability of malignancy either qualitatively by using their clinical judgment and/or quantitatively by using a validated model (Grade 2C).

4.2 Functional Imaging

Most functional imaging of lung nodules is done with PET scan, but other modalities include dynamic contrast-enhanced CT scan, dynamic MRI, and single-photon emission CT (SPECT) scan.

4.2.1 *Dynamic CT Scan:* CT scan with dynamic contrast enhancement is rarely used in the United States, yet it is highly sensitive (albeit nonspecific) for identifying malignant nodules.[39] A multicenter study enrolled 356 participants with normal renal function and noncalcified nodules that measured 0.5 to 4 cm in diameter, 48% of which were malignant.[40] With a threshold for enhancement of 15 Hounsfield units (HUs), the sensitivity and specificity of contrast-enhanced CT scan were 98% and 58%, respectively. Absence of lung nodule enhancement was strongly predictive of a benign diagnosis (negative predictive value, 96.5%). Allowing for slight differences in technique, similar results have been reported by others.[41-45] However, later studies highlighted the lack of specificity. Even with the use of novel parameters to measure enhancement, contrast-enhanced CT scan does not reliably discriminate between malignant and active inflammatory or infectious nodules (Appendix S3).[46,47]

4.2.2 *Dynamic MRI and SPECT Scan:* A 2008 meta-analysis summarized results of studies of the diagnostic accuracy of PET scan (22 studies), dynamic CT scan (10 studies), dynamic MRI (six studies), and SPECT scan (seven studies).[48] Pooled estimates of sensitivity ranged from 93% for dynamic CT scan to 94% for dynamic MRI to 95% for both PET and SPECT scans, whereas pooled specificity ranged from 76% for dynamic CT scan to 79% for dynamic MRI to 82% for both PET and SPECT scans. Comparison of summary receiver operating characteristic curves showed that there were no significant differences in accuracy among all four modalities.

4.2.3 *PET Scanning With Fluorodeoxyglucose:* The 2008 meta-analysis[48] yielded an estimate for the sensitivity of PET scan that was somewhat higher (95%) than the estimate of 87% that we reported in the second edition of these guidelines.[6,39] Among more recent studies identified by our updated literature review, estimates of sensitivity ranged from 72% to 94% (Table S7).[49-54]

A limitation of most studies of diagnostic accuracy is the use of a single threshold for distinguishing malignant from benign nodules. A prospective study of 344 US veterans with lung nodules addressed this limitation by reporting LRs ratios for five different categories of PET results.[55] In this study, LRs for definitely benign, probably benign, indeterminate, probably malignant, and definitely malignant PET results were 0.03 (95% CI, 0.01-0.12), 0.15 (95% CI, 0.09-0.25), 1.01 (95% CI, 1.00-1.02), 3.2 (95% CI, 1.9-5.3), and 9.9 (95% CI, 5.4-18.3), respectively, confirming the intuition that greater degrees of fluorodeoxyglucose (FDG) uptake are more strongly associated with malignancy. In this study, FDG uptake that was slightly greater than that of the mediastinal blood pool was considered to be probably malignant, whereas substantially greater uptake was considered to be definitely malignant. Although indeterminate findings on PET scan did not help to distinguish malignant from benign nodules, very few participants (1%) had indeterminate findings.

Some have proposed using dual time point measurements of FDG uptake to improve diagnostic accuracy (Table S8). However, a systematic review of 816 patients with 890 nodules in 10 studies concluded that dual time point measurement was no better than single time point measurement.[56] In this study, the pooled sensitivity and specificity of dual-time FDG-PET scan for identifying malignancy were 85% (95% CI, 82%-89%) and 77% (95% CI, 72%-81%), respectively.

False-negative findings on PET scan can be seen in patients with less metabolically active tumors, including lepidic-predominant adenocarcinomas (minimally invasive or in situ), mucinous adenocarcinomas, and carcinoid tumors. False-positive findings often are the result of infections or inflammatory conditions, including (but not limited to) endemic mycoses, TB,

rheumatoid nodules, and sarcoidosis. Paradoxically, false-positive PET scan results can sometimes be helpful because they alert the clinician to the presence of an active infectious or inflammatory condition that might require specific treatment. In some circumstances, FDG-PET scan can be helpful in directing tissue biopsy. As a metabolic biopsy tool, PET scan can identify which lesions or portions of lesions are metabolically active and most likely to yield a definitive tissue result.

Use of FDG-PET scanning may be most cost-effective when clinical pretest probability and CT scan results are discordant, especially when pretest probability is relatively low and CT image characteristics are indeterminate (ie, not clearly benign).[57] Among patients with indeterminate nodules (by CT scan) and high pretest probability, negative PET scan results do not reliably exclude malignancy. However, FDG uptake in the primary tumor has been shown to be inversely correlated with survival,[58,59] and patients with nonhypermetabolic malignant tumors may have a favorable prognosis, even when definitive surgical treatment is delayed by a period of observation as long as 238 days.[60,61] Hence, patients with solid nodules and negative (nonhypermetabolic) PET scan results are believed to require continued surveillance for at least 2 years to confirm benignity. An even more cautious approach would be to perform needle biopsy in high-probability tumors with negative PET scan results.

Integrated PET/CT scanners combine CT and FDG imaging capability in a single patient gantry, facilitating the precise localization of areas of FDG uptake to normal structures or abnormal soft tissue masses. Of three studies that compared dedicated PET scan with integrated PET/CT scan for pulmonary nodule characterization (Table S9), integrated PET/CT scan was slightly more accurate in two of them, but none of the studies compared integrated PET/CT scan with standard care (side-by-side interpretation of dedicated PET scan and dedicated CT scan).[62-64]

Although we view nodule characterization and lung cancer staging as separate indications for PET scanning, we favor PET scan over other functional imaging modalities for nodule characterization in part because PET scan often provides additional information about stage among individuals with malignant nodules. Recommendations about the use of PET scanning for staging are described by Silvestri et al[65] in the "Methods for Staging Non-small Cell Lung Cancer" article in the ACCP Lung Cancer Guidelines.

Although exposure to ionizing radiation from dedicated FDG-PET imaging is at least moderate (about 5-7 mSv), the addition of integrated CT scanning for purposes of attenuation correction and anatomic correlation results in doses that are much higher, especially if a full diagnostic CT scan is performed (about 10-25 mSv).[66] The widespread belief that PET/CT imaging is without risk is not correct.

4.2.4 Recommendations

4.2.4.1. In the individual with a solid, indeterminate nodule that measures ≥ 8 mm in diameter and low to moderate pretest probability of malignancy (5%-65%), we suggest that functional imaging, preferably with PET, should be performed to characterize the nodule (Grade 2C)**.**

4.2.4.2. In the individual with a solid, indeterminate nodule that measures ≥ 8 mm in diameter and a high pretest probability of malignancy (≥ 65%), we suggest that functional imaging should not be performed to characterize the nodule (Grade 2C)**.**

Remark: PET may be indicated for pretreatment staging among those patients with nodules in whom malignancy is strongly suspected or confirmed.

4.3 Management Strategies

Once imaging tests have been performed, management alternatives include surgical diagnosis, nonsurgical biopsy, and surveillance with serial CT scans. Each approach has advantages and disadvantages (Fig 4). Surgery is the diagnostic gold standard and the definitive treatment of malignant nodules, but surgery should be avoided in patients with benign nodules. Nonsurgical biopsy often is used to establish a specific benign or malignant diagnosis, but biopsy is invasive, potentially risky, and frequently nondiagnostic. CT scan surveillance avoids unnecessary surgery in patients with benign nodules, but surveillance delays diagnosis and treatment in cases of malignancy. A decision analysis found that the choice of strategy was a close call across a range of probabilities for malignancy.[67] In this analysis, surveillance was favored when the probability of malignancy was ,3%, and surgical diagnosis was preferred when the probability was ≥ 68%. Biopsy was the recommended strategy when the probability of malignancy fell between 3% and 68%. A management algorithm that is based in part on this analysis and a subsequent cost-effectiveness analysis[57] is presented in Figure 1. More-specific recommendations are outlined next.

4.4 Shared Decision-Making and Patient Preferences

Because different strategies are associated with similar expected outcomes, individual preferences

Figure 4. [Section 4.3] Balance sheet of pros and cons of alternatives for evaluation and management of pulmonary nodule.

Procedure	Potential Benefits		Potential Harms	
	Outcome	% Frequency	Outcome	% Frequency
Surgical wedge resection	• Prompt, definitive diagnosis	96-100	• Physical complications	5
	• Avoid inconvenience and potential complications of nonsurgical biopsy, if malignant		Persistent air leak	3-5
			Pneumonia	1-8
			Death	0.5
	• Reassurance if specific benign diagnosis established			
	• Proceed to lobectomy if frozen section reveals malignancy		• Unnecessary surgery if nodule turns out to be benign disease	Varies
	• Acquisition of tissue for molecular testing		• Uncertain benefits of surgery if very-slow-growing tumor	
Bronchoscopy with biopsy	• Definitive preoperative cancer diagnosis in many cases		• Physical complications	
			Bleeding	2-5
	Fluoroscope-guided	~ 30	Any pneumothorax	2-4
	EBUS, ENB ± VBN guided	60-90	Death	<<1
	• Reassurance if specific benign diagnosis established		• May still require surgery if biopsy result is nondiagnostic or shows cancer	
	• Acquisition of tissue for molecular testing		• False negative biopsy results	30-70
			• False positive biopsy results	Rare
CT scan-guided needle lung biopsy	• Definitive preoperative cancer diagnosis in many cases		• Physical complications	
			Bleeding	1
	≤ 15 mm	~ 70-80	Any pneumothorax	15
	> 15 mm	~ 90	Pneumothorax needing chest tube	6-7
			Death	<<1
	• Reassurance if specific benign diagnosis established		• May still require surgery if biopsy is non-diagnostic or shows cancer	
	• Acquisition of tissue for molecular testing		• False negative	10-30
			• False positive	Rare
Radiologic surveillance (serial CT ± PET scans)	• Avoid physical complications		• Radiation exposure	
	• Discovering other incidental findings that are clinically important		• Other incidental findings that prompt evaluation but turn out to be of little clinical significance	
			• Psychologic toll of uncertainty (eg. moderate to severe distress)	24
			• Overdiagnosis of indolent cancers	
			• Delayed cancer diagnosis and treatment, with uncertain effect on outcomes	
No further evaluation	• Avoid physical complications		• Psychologic toll of uncertainty	
	• Avoid radiation exposure		• Delayed or missed cancer diagnosis	
	• Avoid overdiagnosis of indolent cancers that do not need treatment			

EBUS5endobronchial ultrasound; ENB5electromagnetic navigation bronchoscopy; VBN5virtual bronchoscopy navigation.

should be elicited and used to guide decisions. Some individuals may be uncomfortable with adopting a strategy of surveillance when told that a potentially cancerous lung nodule is present. Others are similarly risk averse about undergoing surgery unless they are certain that cancer is present.[68] All individuals with lung nodules should be provided with an estimate of the probability of cancer and informed about the specific risks and benefits associated with alternative management strategies. Clinicians should elicit preferences for management and be sensitive to the preferred participatory decision-making style of the patient.[69]

4.4.1 Recommendation

4.4.1.1. In the individual with a solid, indeterminate nodule that measures ≥ 8 mm in diameter, we recommend that clinicians discuss the risks and benefits of alternative management strategies and elicit patient preferences for management (Grade 1C).

4.5 CT Scan Surveillance

In some individuals with lung nodules, surveillance with serial imaging tests may be used as a diagnostic

tool. When this strategy is used, detection of growth strongly suggests malignancy, and surgical resection should be performed in patients who are operative candidates. However, very rapidly growing nodules are commonly infectious or inflammatory, necessitating estimation of the growth rate. The growth rate typically is expressed in terms of the doubling time, or the time it takes for the nodule to double in volume. Because the volume of a sphere equals $4\pi r^3/3$, one doubling in tumor volume corresponds approximately to an increase in nodule diameter of 26%. The doubling time can be calculated by using the formula, $dt = (t \times \log 2)/[\log(d_2/d_1)]$, where dt = doubling time in days, t = time in days between radiographs, d_2 = diameter of the nodule at the time of the current radiograph, and d_1 = diameter of the nodule at the time of the previous radiograph.[70]

Two-year radiographic stability is strong presumptive evidence of a benign cause because malignant solid nodules typically double in volume within 400 days.[71,72] Longer duration follow-up is advisable for ground glass nodules, which generally have longer volume doubling times (VDTs) when malignant.

Surveillance is virtually always performed with CT scanning, which is more sensitive than chest radiography for detecting growth. Although it may be possible to detect growth on serial chest radiographs when the nodule is large ($\geq$ 1.5-2 cm) and has sharp, clearly demarcated borders, the surveillance strategy is seldom used for nodules of this size because of the relatively high probability of malignancy. For solid, indeterminate nodules measuring $\geq$ 8 mm in diameter, the optimal time interval between imaging tests has not been determined, but standard practice is to obtain follow-up CT scans at about 3 to 6, 9 to 12, and 18 to 24 months.[73] Less-frequent follow-up is indicated in patients with smaller nodules, as discussed in subsequent sections.

The advantage of the surveillance strategy in avoiding unnecessary invasive procedures among individuals with benign nodules is weighed against the disadvantage of delaying diagnosis and treatment among patients with malignant nodules. Depending on the growth rate and metastatic potential of the nodule and the length of surveillance, some malignant tumors will progress from resectable to unresectable disease during the observation period, and opportunities for surgical cure will be missed. Empirical data relevant to the hazard of delay are scarce, although a Scottish study found that maximum cross-sectional tumor area increased by $\geq$ 50% in almost 25% of patients who had delays in radiotherapy treatment lasting between 18 and 131 days.[74] In contrast, most studies of timeliness of care in lung cancer did not detect an association between timeliness and survival.[75,76] Nevertheless, the surveillance strategy should be avoided when the clinical probability of cancer is moderate to high; it is most appropriate in individuals with a very low probability of malignancy and in those who are at high risk for complications of surgical resection and nonsurgical biopsy.

Methods to detect growth on serial CT scans are evolving rapidly (Appendix S4). Manual measurements of diameter are limited by poor reliability and accuracy. In one study of 63 patients with lung nodules and 93 pairs of CT scans, manual and electronic measures of diameter and cross-sectional area incorrectly assessed the presence or absence of growth in 27% to 37% of CT scan pairs compared with a reference standard of manual volumetric measurement.[77]

Measurement of diameter, therefore, is likely to be supplanted by measurement of volume or mass, but available studies are not yet conclusive, being limited by small samples and retrospective, uncontrolled designs. For example, a small retrospective study of 63 participants (including only 11 participants with malignant nodules) used volumetric software to measure nodules on CT scans performed a median of 3.7 months apart and found that a threshold VDT of $\geq$ 500 days had a sensitivity of 91% and a specificity of 90% for identifying malignancy.[78] In another small study of 13 malignant ground glass nodules detected by screening over a mean time of 33 months between the first and last CT scans, diameter increased by 53%, volume by 202%, and mass by 254%, with measurements of mass having the largest signal-to-noise ratio, suggesting that measurements of volume or mass may improve detection of growth.[79] Finally, in a study of 69 patients with 87 nodules (92% solid), volumetric measurement of growth changed the management decision from observation to biopsy in seven patients, although only three of these patients were proven to have lung cancer, with VDTs ranging from 347 to 670 days in these three cases.[80]

However, size measurements are fraught with error. Various measurement methods of solid nodules all have false-positive and false-negative assessments of growth.[81] There is poor interobserver and intraobserver consistency for size differences of $\leq$ 1.5 to 2 mm.[82,83] In a study of 100 patients with 233 screening-detected benign nodules all measuring at least 4.8 mm in diameter,[84] variability in automated volumetric measurements between nodules seen on CT scans performed at baseline, 3 months, and 12 months was ±27%, and 70% of measurements had volume differences $\geq$ 10%. Even in phantom studies, there is an error rate of about 20% in determining the presence or absence of one volume doubling for 5-mm nodules with a slice thickness of 2.5 mm.[82] Finally, emerging data suggest that the rate of growth may not be constant and that a decrease in size is observed

in about 20% of malignant lesions.[85] These issues need to be better understood and standards developed.

Doubling times for malignant nodules are highly variable, but solid nodules usually have doubling times between 20 and 400 days. Because of this, 2-year radiographic stability of a solid nodule strongly implies a benign etiology.

4.5.1 Recommendations

4.5.1.1. In the individual with a solid, indeterminate nodule that measures ≤ 8 mm in diameter, we suggest surveillance with serial CT scans in the following circumstances (Grade 2C)**:**

- **When the clinical probability of malignancy is very low (≤ 5%)**
- **When clinical probability is low (≤ 30% to 40%) and the results of a functional imaging test are negative (ie, the lesion is not hypermetabolic by PET or does not enhance ≥ 15 HUs on dynamic contrast CT), resulting in a very-low posttest probability of malignancy**
- **When needle biopsy is nondiagnostic and the lesion is not hypermetabolic by PET**
- **When a fully informed patient prefers this nonaggressive management approach.**

Remark: CT surveillance of solid nodules ≤ 8 mm should use low-dose, noncontrast techniques with thin sections through the nodule of interest.

4.5.1.2. In the individual with a solid, indeterminate nodule that measures ≤ 8 mm in diameter who undergoes surveillance, we suggest that serial CT scans should be performed at 3 to 6, 9 to 12, and 18 to 24 months, using thin sections and noncontrast, low-dose techniques (Grade 2C)**.**

Remark: Serial CT scans should be compared with all available prior studies, especially the initial (index) CT scan.

Remark: Where available, manual and/or computer-assisted measurements of area, volume, and/or mass may facilitate early detection of growth.

4.5.1.3. In the individual with a solid, indeterminate nodule that shows clear evidence of malignant growth on serial imaging, we recommend nonsurgical biopsy and/or surgical resection unless specifically contraindicated (Grade 1C)**.**

Remark: Solid nodules that decrease in size but do not disappear completely should be followed to resolution or lack of growth over 2 years.

4.6 Nonsurgical Biopsy

Options for nonsurgical tissue diagnosis include CT scan-guided TTNB and bronchoscopy guided by fluoroscopy, endobronchial ultrasound (EBUS), electromagnetic navigation bronchoscopy (ENB), and virtual bronchoscopy navigation (VBN).

4.6.1 Transthoracic Needle Biopsy: TTNB of the pulmonary nodule usually is performed under CT scan guidance. In general, the sensitivity of TTNB depends on the size of the nodule, the size of the needle (especially for identifying lymphoma or benign disease), the number of needle passes, and the presence of onsite cytopathologic examination. In our previous review,[39] we identified 11 studies of TTNB performed between 1998 and 2003. In these studies, the prevalence of malignancy was high (median, 75%; range, 63%-85%). Nondiagnostic results were seen in 4% to 41% of cases (median, 20.5%), but specific benign or malignant results were nearly always correct (although not all malignant diagnoses were confirmed surgically).

Our updated literature search identified 11 additional studies of TTNB for pulmonary nodule diagnosis performed between 2005 and 2011 (Tables S10-S13). Once again, the prevalence of malignancy was high (median, 68%; range, 46%-83%), and the frequency of nondiagnostic results was highly variable, ranging from ≤ 1% to 55%, although the median value was lower than what we found previously (6%). In most studies, sensitivity for identifying malignancy was >90%, but it was somewhat lower (70%-82%) in three studies that analyzed results for patients with nodules measuring <15 mm in diameter.[62,86,87] More importantly, a sensitivity of 90% in a high-prevalence population (about 70%) translates to a risk of nondiagnostic results in about 20% of individuals with malignant nodules.

Our search did not identify any randomized controlled trials comparing TTNB with other approaches, but a 2002 study used case vignettes from 114 patients with solitary nodules (71% malignant) to determine the frequency with which TTNB results changed management.[88] In this study, the addition of TTNB results to clinical history and chest CT scan findings reduced the frequency of missed surgical cure from 10% to 7% and reduced the frequency of unnecessary surgery for a benign lesion from 39% to 15%.

Complications of TTNB include pneumothorax and hemorrhage. In a population-based study of all TTNB procedures performed in California, Florida, Michigan, and New York in 2006, the risk of hemorrhage was low (1%), but the risks of any pneumothorax (15%) and pneumothorax requiring chest tube insertion (6.6%) were substantial.[89] In this study, risk

factors for pneumothorax included age 60 to 69 years, tobacco use, and COPD. In other single-center studies, risk factors for pneumothorax included older age,[90] smaller lesion size,[91-94] deeper location,[90-96] the need to traverse fissures,[91] the presence of emphysema,[95,96] and the number of needle punctures.[97]

Use of needle biopsy is probably most appropriate when there is discordance among the clinical probability of cancer, imaging test results, patient preferences, and risk of surgical complications, as described in recommendation 4.6.2.1.1. It is important to emphasize that a nondiagnostic needle biopsy result does not rule out the possibility of malignancy.

4.6.2 *Bronchoscopy:* Until recently, bronchoscopy played a limited role in pulmonary nodule management outside investigational settings. In older studies, the sensitivity of fluoroscopy-guided bronchoscopy with transbronchial biopsy (TBB) for identifying malignant nodules measuring ≥ 2 cm in diameter ranged from 5% to 76% (median, 31%).[98] The likelihood of obtaining a specific benign diagnosis is even lower. However, the presence of an air bronchogram in a pulmonary nodule is associated with a higher diagnostic yield, especially if this provides a specific road map to the bronchial location.[99,100]

Newer techniques for bronchoscopic guidance include radial EBUS (Appendix S5), ENB (Appendix S6), and VBN. EBUS facilitates bronchoscopic sampling of smaller peripheral nodules. A recent systematic review identified 13 studies that reported the sensitivity of EBUS-TBB for identifying malignancy in 1,090 patients with peripheral lung lesions.[101] For lesions of any size, pooled sensitivity was 0.73 (95% CI, 0.70-0.76); sensitivity was similar when pooled across seven studies that enrolled 580 patients with nodules measuring ≥ 25 mm in diameter (0.71; 95% CI, 0.66-0.75). Studies were limited by low scores for study quality, inconsistent results, and the indirectness that characterizes most studies of diagnostic accuracy (Fig 5, Tables S14-S16).

Two small randomized trials compared EBUS-TBB with conventional TBB (Fig 5). In one study, the sensitivity of EBUS-TBB for identifying malignant nodules measuring < 20 mm was markedly greater than that for conventional TBB (71% vs 23%).[103] However, a subsequent study reported a sensitivity of only 11% for EBUS-TBB compared with 31% for conventional TBB[102] perhaps because few of the participating bronchoscopists had experience with EBUS. A pooled analysis of complication rates from both trials neither confirmed nor excluded differences between the two tests (relative risk, 0.49; 95% CI, 0.02-14.7) (Fig S1).

ENB shows promise as another tool for guiding biopsy of peripheral nodules.[104,105] Our literature review identified 10 studies that reported the sensitivity of ENB-guided TBB for the identification of malignancy in peripheral lung lesions, including four studies that described results for nodules measuring ≥ 2 cm (Tables S17-S19). Among the latter studies, diagnostic yield ranged from 44% to 75% (median, 68.5%). Across all 10 studies, the risk of pneumothorax ranged from 0% to 7.5% (median, 2.2%). Studies were limited by small sample sizes, uncertain representativeness of the study populations, and retrospective uncontrolled design.

A small (n 5 118) randomized controlled trial compared EBUS-TBB, ENB, and EBUS-TBB plus ENB for diagnostic yield in the absence of fluoroscopic guidance.[106] For peripheral lesions of any size, diagnostic yield was higher for the combined procedure (88%) than for EBUS-TBB (69%) or ENB (59%) alone. Results were similar when the analysis was restricted to nodules measuring 20 to 30 mm in diameter or nodules measuring ≥ 20 mm in diameter.

More recently, a randomized controlled trial from three centers in Japan compared VBN-assisted EBUS with nonassisted EBUS for diagnostic yield among 199 individuals with nodules measuring up to 30 mm in diameter.[107] In this trial, diagnostic yield was higher for the VBN-assisted procedure (80%) than for the unassisted procedure (67%).

A recent meta-analysis identified 39 studies of bronchoscopy with biopsy guided by radial EBUS (20 studies), ENB (11 studies), guide sheath (10 studies), ultrathin bronchoscopy (11 studies), or VBN (10 studies).[108] Most studies were prospective but limited by small samples. Across all studies, the pooled diagnostic yield was 70% (95% CI, 67%-73%), with slightly more favorable results for guide sheath (73%) and slightly less favorable results for ENB (67%). Heterogeneity in study results was identified but not entirely explained, although diagnostic yield for nodules measuring < 20 mm in diameter (61%; 95% CI, 54%-68%) was substantially lower than that for nodules measuring ≥ 20 mm in diameter (82%; 95% CI, 78%-86%). Across 24 studies that reported adverse events, the pooled risk of pneumothorax was 1.6%, and the risk of pneumothorax requiring chest tube placement was 0.7%.

For individuals who choose to pursue nonsurgical biopsy, the decision to perform CT scan-guided TTNB; conventional bronchoscopy; or bronchoscopy guided by EBUS, ENB, or VBN depends on multiple factors. CT scan-guided TTNB is preferred for nodules located in proximity to the chest wall or for deeper lesions provided that fissures do not need to be traversed and there is no surrounding emphysema. Bronchoscopic techniques are favored for nodules located in proximity to a patent bronchus and in individuals who are at high risk for pneumothorax following

Figure 5. [Section 4.6.2] EBUS-TBB compared with TBB guided by fluoroscopy for patients with peripheral lung nodules.

				Anticipated effects	
				TBB guided by fluoroscopy (95% CI)	**EBUS-TBB**
	(2 RCTs)			**Pooled sensitivity= 31.1%** (95% CI 21.1% to 42.0%)	**Pooled sensitivity= 41.1%** (95% CI 0% to 96.5%)
Sensitivity of EBUS-TBB	580 (7 studies of accuracy[e])	⊕⊖⊖⊖ **VERY LOW**[b,c,d,f]	N/A	N/A	**Pooled sensitivity= 71.0%** (95% CI 0.66 to 0.75)
			RR 0.49	**70 per 1000**	**35 fewer per 1000** (from 68 fewer to 953 more)

GRADE Working Group grades of evidence: **High quality** indicates that further research is very unlikely to change our confidence in the estimate of effect; **moderate quality**, further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate; **low quality**, further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate; and **very-low quality**, we are very uncertain about the estimate.

From Steinfort et al,[101] Roth et al,[102] and Paone et al.[103]

GRADE = working group grades of evidence; RCT = randomized controlled trial; RR = relative risk; TBB = transbronchial biopsy. See Figure 3 legend for expansion of other abbreviation.

[a]Approximately 10% of patients were excluded from postrandomization.

[b]Statistically significant unexplained heterogeneity between studies.

[c]Surrogate outcome.

[d]Pooled effect imprecise and consistent with either benefit or no effect.

[e]Seven of 16 studies in systematic review provided data for nodules measuring < 25 mm.

[f]Mean QUADAS (quality assessment of studies of diagnostic accuracy) score 3.3 (maximum, 6-14).

TTNB. In most other situations, operator experience should guide the decision.

4.6.2.1 Recommendation

4.6.2.1.1. In the individual with a solid, indeterminate nodule that measures ≥ 8 mm in diameter, we suggest nonsurgical biopsy in the following circumstances (Grade 2C)**:**

- **When clinical pretest probability and findings on imaging tests are discordant**
- **When the probability of malignancy is low to moderate (~10% to 60%)**
- **When a benign diagnosis requiring specific medical treatment is suspected**
- **When a fully informed patient desires proof of a malignant diagnosis prior to surgery, especially when the risk of surgical complications is high.**

Remark: The type of biopsy should be selected based on nodule size, location, and relation to a patent airway; the risk of complications in the individual patient; and available expertise.

4.6.3 *Surgical Diagnosis:* Surgical resection is both the gold standard for diagnosis and the definitive treatment of a malignant nodule. The decision to pursue surgical diagnosis must take into account the benefits of definitive diagnosis and treatment when compared with the surgical risk. Video-assisted thoracic surgery (VATS), thoracotomy, and mediastinoscopy may be used alone or in combination, depending on the clinical circumstances. If the nodule proves to be a primary lung cancer, diagnosis, staging, and therapeutic resection often are completed in a single operative procedure.

Thoracoscopic wedge resection is the strongly preferred diagnostic approach for nodules. Although less invasive and almost certainly less morbid than open thoracotomy, data on complications of VATS diagnostic wedge resection are sparse. In two small older studies, there were no fatal complications; nonfatal complications occurred in about 5% of patients.[109,110] More recent reports are difficult to interpret because they combine results for diagnostic VATS with VATS lobectomy, often in patients with severe comorbid conditions.

Nodules that are small in size (≤ 1 cm), deep in location, and subsolid in attenuation can pose a technical challenge because it may be difficult to find such nodules by digital palpation. Localization techniques to increase diagnostic yield during thoracoscopy include hook and wire, radioguidance, methylene blue, percutaneous microcoils, ultrasound, and fluoroscopy. In a recent review of methods to localize small nodules, the sensitivity of finger palpation was very poor in one study (≤ 30%) but excellent in another (88%).[111] Hook-and-wire techniques had a sensitivity of 58% to 97%, with wire dislodgment being the largest source of failure. Technetium-99 radioguidance and fluoroscopic guidance with contrast material had high sensitivity with few complications. Ultrasonography had a sensitivity of 93% to 100% but is believed to be operator dependent.

The diagnosis is most often established by intraoperative consultation with pathology. Frozen section analysis is sensitive and specific for diagnosis of malignancy; however, the technique has limitations the surgeon should understand. In one study, the sensitivity for identifying malignancy was 87% for nodules that measured ≤ 1.1 cm in diameter and 94% for nodules that measured between 1.1 and 1.5 cm.[112] The technique has limitations in distinguishing minimally invasive adenocarcinoma or adenocarcinoma in situ (AIS) from atypical adenomatous hyperplasia (AAH) and in establishing a specific cell type in non-small cell carcinoma. It is limited in recognizing small peripheral carcinoid tumors. Lesions measuring ≤ 5 mm should probably not be used for frozen section analysis unless there is other material available for permanent studies.[112]

For the surgical candidate with a nodule shown to be non-small cell lung cancer, lobectomy and systematic sampling of mediastinal lymph nodes is the standard of care for complete oncologic resection and staging.[113] Minimally invasive techniques are increasingly preferred for lobectomy. Several large contemporary studies reported risks of fatal and nonfatal complications. Among nearly 6,000 individuals from the Society of Thoracic Surgeons database who underwent lobectomy between 1999 and 2006, of whom 30% underwent thoracoscopic procedures, the reported 30-day mortality was about 2%.[114] Among smaller numbers of individuals who underwent bilobectomy and pneumonectomy in this study, the risks of fatal complications were 4% and 6.2%, respectively. More recently, data from the Nationwide Inpatient Sample showed in-hospital mortality to be 3.1% among 12,860 individuals who underwent open lobectomy and 3.4% among 759 individuals who underwent VATS lobectomy.[115] An observational study of . 2,500 propensity-matched patients from the Society of Thoracic Surgeons database reported a higher percentage of patients who were free of complications among those who underwent thoracoscopic lobectomy compared with open lobectomy (74% vs 65%).[116]

For individuals with marginal cardiac performance or limited pulmonary reserve, sublobar resection can be considered acceptable treatment, although lobectomy has been the standard of care for medically fit

populations. In the only randomized trial comparing lobectomy with lesser resection, there was an increase in the risk of locoregional recurrence with sublobar resection.[117,118] That trial completed accrual in 1988, and advances in radiologic detection of small nodules and increased understanding of varied tumor biology have led to a resurgence of interest in limited resection for stage I non-small cell lung cancer. Accordingly, a randomized trial of lobectomy vs sublobar resection for biopsy specimen-proven, node-negative tumors measuring ≤ 1 cm is ongoing.

An oncologic resection is not complete without staging the mediastinum. Recommendations for intraoperative staging can be found elsewhere.[65]

4.6.3.1 Recommendations

4.6.3.1.1. In the individual with a solid, indeterminate nodule that measures ≥ 8 mm in diameter, we suggest surgical diagnosis in the following circumstances (Grade 2C)**:**

- **When the clinical probability of malignancy is high (≥ 65%)**
- **When the nodule is intensely hypermetabolic by PET or markedly positive by another functional imaging test**
- **When nonsurgical biopsy is suspicious for malignancy**
- **When a fully informed patient prefers undergoing a definitive diagnostic procedure.**

4.6.3.1.2. In the individual with a solid, indeterminate nodule measuring > 8 mm in diameter who chooses surgical diagnosis, we recommend thoracoscopy to obtain a diagnostic wedge resection (Grade 1C)**.**

Remark: Use of advanced localization techniques or open thoracotomy may be necessary when resecting small or deep nodules.

5 Solid Nodules Measuring < 8 mm in Diameter

On the basis of observations from lung cancer screening trials, the attenuation of nodules may be characterized as solid or subsolid. Subsolid nodules can be further classified as part-solid or pure ground glass (defined as focal densities in which underlying lung morphology is preserved). Part-solid and ground glass nodules are discussed subsequently. Solid nodules are the most frequently encountered type but least likely to be malignant among the three types.[119,120]

Small, solid nodules can be solitary or nonsolitary and are usually detected incidentally on a CT scan that has been ordered for some other reason. As is true for larger nodules, the likelihood of malignancy depends on patient risk factors, nodule size, and morphology.

5.1 *Predictors of Malignancy*

Patient characteristics have been incompletely studied as predictors of malignancy among individuals with solid nodules measuring <8 mm in diameter. In the Lung Screening Study, abnormal findings on a single low-dose CT screening examination were more common in current smokers and individuals who were aged at least 65 years.[121] The likelihood of malignancy is probably highest in current smokers and lowest in nonsmokers who have nodules that are comparable in size. Extrapolation from studies in patients with larger nodules suggests that the risk of malignancy probably increases with age.[26-28]

5.1.1 Size: Studies of CT screening in volunteers at risk for lung cancer confirmed a strong association between nodule diameter and the likelihood of malignancy.[39] Data from baseline screening in US trials of low-dose CT imaging showed that the probability of malignancy is extremely low (≤ 1%) in prevalent nodules that measure ≤ 5 mm in diameter.[121-123] For nodules that measure 5 to 9 mm in diameter, the prevalence of malignancy ranges from 2.3% to 6%.[121,123] In one Japanese study, the prevalence of malignancy in subcentimeter nodules was ≥ 20%, which is considerably higher than in the US studies.[124]

Similar results have been reported in nonscreened populations evaluated by CT imaging. One retrospective review of 3,446 consecutive chest CT scans at a single institution identified 87 patients with incidentally detected lung nodules measuring ≤ 10 mm in diameter and definitive 2-year follow-up. Although 10 (11%) of these nodules were malignant, nine proved to be metastases in patients with known extrathoracic malignancies (who comprised 56% of the study population).[125] In a retrospective review of 414 patients with no history of neoplasm, infection, fibrosis, or immune deficiency and one or more noncalcified lung nodules measuring ≤ 5 mm, none of the nodules were observed to grow over 3 to 24 months of follow-up.[126] The upper boundaries of the 95% CIs for the probability of growth in these small nodules were 0.9%, 1.0%, and 1.3% at 3, 6, and 12 months, respectively.

5.2 *Management Strategies*

The optimal approach to the evaluation and management of solid nodules measuring <8 mm remains problematic. Small nodules are difficult to biopsy, and

although evidence from a few small studies is decidedly mixed,[127-129] consensus holds that they are not reliably characterized by PET scan. Given the relatively low prevalence of malignancy, the risks of surgical diagnosis usually outweigh the benefits. Accordingly, solid, subcentimeter nodules are typically followed with serial CT scans. The frequency and duration of follow-up is guided by consensus-based recommendations first published by the Fleischner Society (Fig 6) and subsequently endorsed in the second edition of these guidelines.[6,73] Decisions about the frequency and duration of follow-up for patients with small solid nodules need to weigh multiple considerations, including clinical risk factors; nodule size; the variable rate of nodule growth; the limited accuracy of available techniques for establishing growth by cross-sectional and volumetric measurements, especially for nodules that measure ≤ 5 mm in size[81,83,130]; concerns regarding radiation dose[24,131,132]; risk factors for surgical complications; and cost.

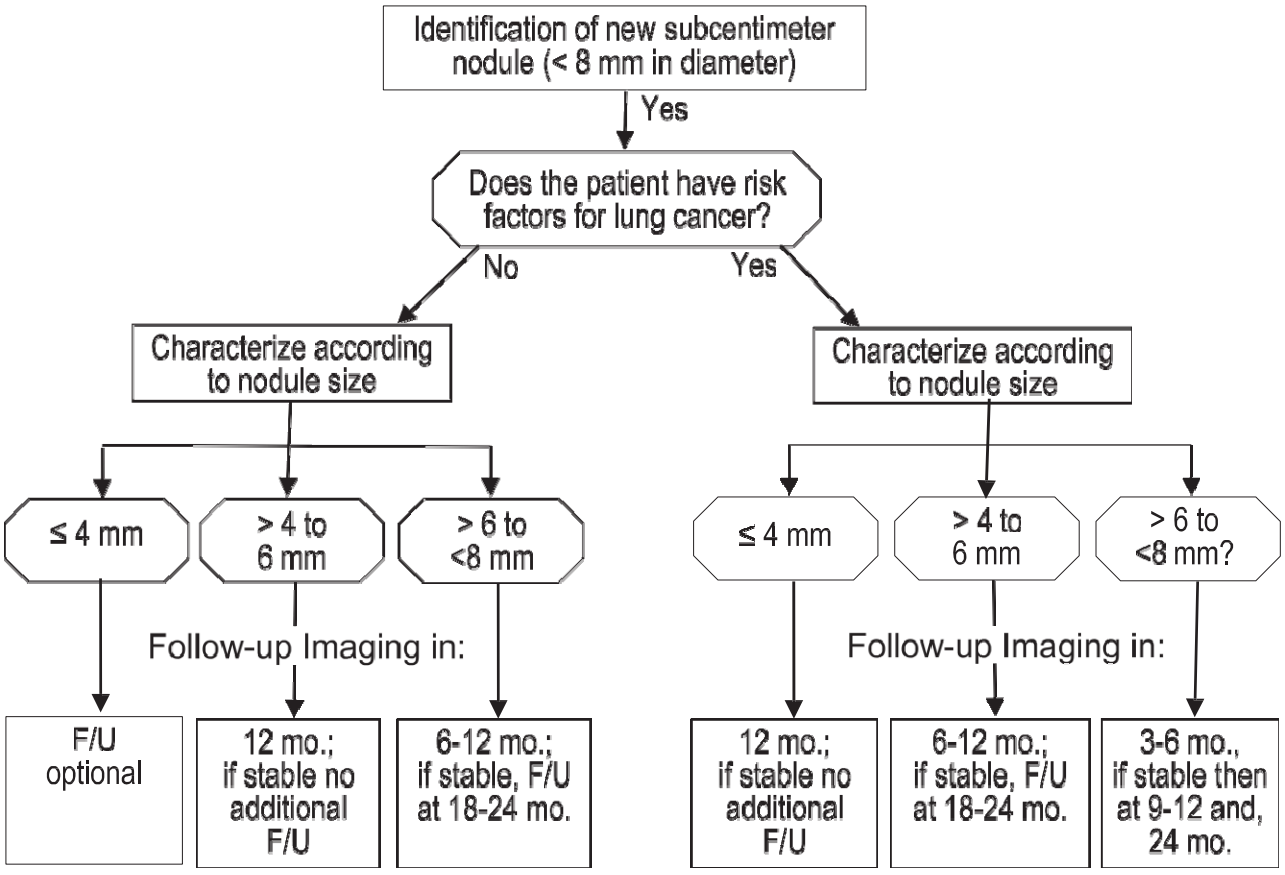
To date, recommendations from the Fleischner Society have not been subjected to formal validation, and limited data suggest that adherence may be suboptimal in some settings.[133,134] Given the absence of new, high-quality evidence, our recommendations for follow-up of solid nodules that measure ≤ 8 mm are unchanged. Recommendations specifically pertain to asymptomatic individuals with no history of extrathoracic malignancy. Once again, we reiterate that follow-up studies should be performed with the lowest possible radiation dose to minimize cumulative radiation exposure in individuals who require multiple follow-up CT examinations.

Recommendations

5.3.1. In the individual with a solid nodule that measures < 8 mm in diameter and no risk factors for lung cancer, we suggest that the frequency and duration of CT surveillance be chosen according to the size of the nodule (Grade 2C)**:**

- **Nodules measuring < 4 mm in diameter need not be followed, but the patient should be informed about the potential benefits and harms of this approach**
- **Nodules measuring ≥ 4 mm to 6 mm should be reevaluated at 12 months without the need for additional follow-up if unchanged**
- **Nodules measuring ≥ 6 mm to 8 mm should be followed sometime between 6 and 12 months and then again at between 18 and 24 months if unchanged.**

Figure 6. [Section 5.2] Management algorithm for individuals with solid nodules measuring ≤ 8 mm in diameter. F/U5follow-up.



Remark: For the individual with multiple small, solid nodules, the frequency and duration of follow-up should be based on the size of the largest nodule.

Remark: CT surveillance of solid nodules < 8 mm should use low-dose, noncontrast techniques.

5.3.2. In the individual with a solid nodule that measures < 8 mm in diameter who has one or more risk factors for lung cancer, we suggest that the frequency and duration of CT surveillance be chosen according to the size of the nodule (Grade 2C)**:**

- **Nodules measuring < 4 mm in diameter should be reevaluated at 12 months without the need for additional follow-up if unchanged**
- **Nodules measuring ≥ 4 mm to 6 mm should be followed sometime between 6 and 12 months and then again between 18 and 24 months if unchanged**
- **Nodules measuring ≥ 6 mm to 8 mm should be followed initially sometime between 3 and 6 months, then subsequently between 9 and 12 months, and again at 24 months if unchanged.**

Remark: For the individual with multiple small, solid nodules, the frequency and duration of follow-up should be based on the size of the largest nodule.

Remark: CT surveillance of solid nodules < 8 mm should use low-dose, noncontrast techniques.

6.0 Subsolid Nodules

In this section, we make recommendations for evaluation and management of asymptomatic individuals with focal, rounded opacities that are subsolid, that is, either nonsolid (pure ground glass) or part solid (with a solid component but ≤ 50% ground glass). Recommendations are predicated on several competing considerations, including the relatively high prevalence of premalignant and malignant disease, uncertainty about the sensitivity of PET scan and needle biopsy, challenges associated with measuring and identifying growth on serial CT scans, and the uncertain prognosis of untreated premalignant disease and AIS.

Among individuals with resected subsolid nodules, the prevalence of premalignant or malignant disease is high, although surgical series may be biased by selection of individuals with greater suspicion for malignancy. As summarized by Detterbeck and Homer,[135] the frequency of AAH, AIS, and invasive adenocarcinoma vary by attenuation characteristics and size. Small (<10 mm), pure ground glass nodules usually represent AAH or AIS; invasive adenocarcinoma is rare.[136-140] The frequency of invasive adenocarcinoma is greater for pure ground glass nodules measuring ≥ 10 mm, reportedly varying from 10% to 50%.[136-139]

AIS and invasive adenocarcinoma are especially prevalent in subsolid nodules that have a large (≥ 50%) solid component, and development of a solid component in a previously nonsolid nodule is strong presumptive evidence of invasive malignancy.[119,137,141-144] As is true for pure ground glass nodules, larger part-solid nodules are more likely to be malignant and invasive than smaller part-solid nodules.[138-140,143,145-147] Clinical, pathologic, radiographic, and molecular features of pulmonary adenocarcinoma and its precursor lesions have been described in a recently revised classification scheme.[148]

Although attenuation characteristics and size are potentially helpful guides to predicting malignancy, some studies have reported counterintuitive results. One small study reported that factors associated with resolution of subsolid nodules detected by screening included larger size, a lobular border, polygonal shape, and partly solid (mixed) attenuation.[19] Another study of nonresolving ground glass nodules, 75% of which were adenocarcinoma (invasive or in situ), found no differences among the size, shape, margin contour, and attenuation characteristics of malignant and benign nodules, although the study was probably underpowered to detect such differences.[136]

6.1 Functional Imaging With PET Scan

Many experts believe that subsolid nodules are not reliably characterized by PET scan,[135,149] but only a few small studies addressed this question. In one study that included 15 nonsolid nodules, PET scan correctly identified only one of 10 malignant nodules and one of five benign nodules.[150] A later study of 68 subsolid nodules reported that the sensitivity of a standardized uptake value of ≥ 1.2 for identifying malignancy was 62%, whereas specificity at this threshold was 80%.[151] In other studies of patients with lepidic growth-predominant adenocarcinoma (bronchioloalveolar cell carcinoma), the sensitivity of PET scan for identifying malignancy ranged from 47%[152] to 60%[153] to 89%.[154] In these studies, sensitivity was higher for mixed and multifocal bronchioloalveolar cell carcinoma. Other studies reported that FDG uptake was inversely correlated with the extent of the lepidic component on pathologic analysis, highlighting the limitations of PET imaging in lepidic-predominant tumors.[155-157]

Although false-negative PET scan results may be more common among individuals with subsolid nodules, absence of FDG avidity portends a favorable

prognosis following surgical resection.[152,157-159] However, it is not certain whether this favorable prognosis extends to patients with nonavid nodules in whom resection is delayed by a period of observation.

6.2 Role of Nonsurgical Biopsy

Few studies have examined the accuracy of nonsurgical biopsy among individuals with subsolid nodules. In a study of 28 such individuals, CT scan fluoroscopy-guided needle biopsy had a sensitivity of 67% for identifying malignancy, although sensitivity was lower for pure ground glass nodules.[160] Similarly, the diagnostic yield of CT scan-guided fine needle aspiration in another study was only 51% for 43 ground glass-predominant nodules compared with 76% for 53 solid-predominant nodules.[161] However, in another study of 50 individuals with subsolid nodules, the sensitivity of CT scan-guided core biopsy was ≥ 90%, regardless of nodule size or extent of the ground glass component.[162] In another small study of 40 individuals with subsolid nodules, the diagnostic yield of CT scan-guided core needle biopsy was 84% (16 of 19), but two of three individuals with nondiagnostic results were subsequently found to have cancer.[163] Hence, although TTNB appears to be less sensitive for subsolid than for solid nodules, it is still potentially useful, particularly for individuals who are at higher risk for surgical complications and those who wish to confirm malignancy before undergoing surgical resection. Although nonsurgical biopsy specimens can confirm the diagnosis of malignancy preoperatively in some cases, it should not be used to exclude malignancy in view of its imperfect sensitivity and limited negative predictive value.

6.3 CT Scan Surveillance

Measurement of subsolid nodules is challenging, and CT scan surveillance is confounded by measurement error, indistinct margins, and long VDTs. Although measurement techniques are improving, both manual and computer-assisted methods remain limited by poor reliability and lack of large-scale validation. Other sources of measurement error include breathing artifact and variable patient positioning.

The slow growth rates of most subsolid malignant nodules have implications for both the frequency and the duration of follow-up. In most cases, observed growth rates for subsolid malignant nodules range between 400 and 800 days, but doubling times as long as 1,500 days have been reported.[164-167] In a small study of 13 subsolid malignant nodules detected by screening, mean times for growth to be detectable (defined as the upper limit of agreement between readers) were 715, 673, and 425 days for measurements of diameter, volume, and mass, respectively.[79] Although development of a solid component often is associated with progression to invasive adenocarcinoma, one study showed that both interreader and intrareader agreement for detection of a solid component were only modest.[79]

Controversy persists regarding how long follow-up should be continued for both part-solid and, especially, pure ground glass nodules.[145,165,168] On the one hand, prognosis appears to be excellent for malignant nodules that are either pure ground glass[138,169,170] or part solid,[171-175] even when treated by sublobar resection, raising the question of whether at least some of these nodules represent indolent cases of lung cancer that may not require treatment (ie, overdiagnosis). On the other hand, measurement challenges and the potential to take on a more-aggressive phenotype argue for greater caution. As a consequence, longer follow-up extending over several years may be appropriate, particularly when there is a history of lung cancer.[176]

6.4 Recommendations for Management

Informal recommendations for the management of subsolid nodules have been published previously,[135,149] and guidelines from the Fleischner Society are forthcoming.[177] The Fleischner Society recommends no follow-up for small (<5 mm) pure ground glass nodules. For larger nonsolid lesions, it recommends an initial follow-up CT scan at 3 months followed by annual follow-up of at least 3 to 5 years. The Fleischner Society views part-solid nodules that persist over 3 months as malignant until proven otherwise, especially when the solid component measures ≥ 5 mm in diameter. All three groups recommend against the use of either PET scan or needle biopsy in most cases, although the Fleischner Society recommends PET scan for part-solid nodules measuring at least 8 mm in diameter.

6.5 Recommendations

6.5.1. In the individual with a nonsolid (pure ground glass) nodule measuring <5 mm in diameter, we suggest no further evaluation (Grade 2C)**.**

6.5.2. In the individual with a nonsolid (pure ground glass) nodule measuring ≥ 5 mm in diameter, we suggest annual surveillance with chest CT for at least 3 years (Grade 2C)**.**

Remark: CT surveillance of nonsolid nodules should use noncontrast techniques with thin sections through the nodule of interest.

Remark: Nonsolid nodules that grow or develop a solid component are often malignant, prompting further evaluation and/or consideration of resection.

Remark: Early follow-up at 3 months may be indicated for nonsolid nodules measuring ≥ 10 mm (followed by nonsurgical biopsy and/or surgical resection for nodules that persist).

Remark: Limited duration or no follow-up may be preferred by individuals with life-limiting comorbidities in whom a low-grade malignancy would be of little consequence or by others who place a high value on avoiding treatment of possibly indolent lung cancer.

6.5.3. In the individual with a part-solid nodule measuring < 8 mm in diameter, we suggest CT surveillance at approximately 3, 12, and 24 months, followed by annual CT surveillance for an additional 1 to 3 years (Grade 2C)**.**

Remark: CT surveillance of part-solid nodules should use noncontrast techniques with thin sections through the nodule of interest.

Remark: Part-solid nodules that grow or develop a solid component are often malignant, prompting further evaluation and/or consideration of resection.

Remark: Limited duration or no follow-up may be preferred by individuals with life-limiting comorbidities in whom a low-grade malignancy would be of little consequence or by others who place a high value on avoiding treatment of possibly indolent lung cancer.

6.5.4. In the individual with a part-solid nodule measuring ≥ 8 mm in diameter, we suggest repeat chest CT at 3 months, followed by further evaluation with PET, nonsurgical biopsy, and/or surgical resection for nodules that persist (Grade 2C)**.**

Remark: PET should not be used to characterize part-solid lesions in which the solid component measures <8 mm.

Remark: Nonsurgical biopsy can be used to establish the diagnosis and/or be combined with wire, radioactive seed, or dye localization to facilitate subsequent resection. A nondiagnostic biopsy result does not exclude the possibility of malignancy.

Remark: Part-solid nodules measuring ≥ 15 mm in diameter should proceed directly to further evaluation with PET, nonsurgical biopsy, and/or surgical resection.

7.0 Individuals With One or More Additional Nodules Detected During Nodule Evaluation

In individuals with known or suspected lung cancer, CT scan will frequently identify one or more additional nodules. Most of these additional nodules are benign. A study from Japan showed that 10% of patients with suspected lung cancer had a second nodule detected during subsequent evaluation, and 60% of these were benign at surgery.[178] In another study, CT scan detected a second indeterminate nodule in 16% of patients with clinically operable stage I to IIIA non-small cell lung cancer.[179] The nodules ranged in size from 4 to 12 mm, and although many of the nondominant nodules were lost to follow-up, ≥ 85% of those with a definite diagnosis were benign.

Screening studies provide additional evidence that patients with a malignant nodule commonly will have additional benign nodules. In an uncontrolled study of CT scan screening in New York, 30% of the participants with cancer identified during baseline (prevalence) screening had one or more additional nodules at the time of detection.[180] None of these were reported to be malignant after follow-up.[181] In the Mayo Clinic screening study, ≥ 50% of the 31 participants with cancer had other nodules detected, and all but one (a carcinoid tumor) were proven to be benign by absence of growth during follow-up.[182] In these studies, the majority of secondary nodules measured ≤ 4 mm, which implies a very low risk of malignancy. Therefore, although the likelihood of finding one or more additional nodules increases with the use of smaller slice thickness on CT scan, the majority of additional nodules will be benign.

When confronted with one or more additional nodules during nodule evaluation, it is prudent to consider each nodule individually rather than assuming that the additional nodules are either metastatic or benign. Preoperative PET scanning may help in the decision of whether more than one nodule is likely malignant and guide further evaluation, although many of these nodules will be too small to be reliably characterized by PET scan. Above all, candidates for curative treatment with known or suspected malignant nodules who have one or more additional nodules present should not be denied curative therapy unless metastasis is confirmed by histopathology. A more-detailed discussion is provided by Kozower et al[183] in the in "Special Treatment Issues in Non-small Cell Lung Cancer" article of the ACCP Lung Cancer Guidelines.

7.1 Recommendation

7.1.1. In the individual with a dominant nodule and one or more additional small nodules, we suggest that each nodule be evaluated individually and curative treatment not be denied unless there is histopathological confirmation of metastasis (Grade 2C)**.**

Remark: The classification and appropriate treatment of patients with more than one pulmonary focus of lung cancer is difficult and requires multidisciplinary consideration.

8.0 Conclusions and Recommendations for Research

The pulmonary nodule is increasingly common and remains a vexing problem. Individuals with solid nodules measuring ≥ 8 mm should be managed by reviewing old imaging studies; estimating the probability of malignancy; performing imaging tests to better characterize the nodule; evaluating the risks associated with various management alternatives; and eliciting patient preferences for CT scan surveillance, nonsurgical biopsy, or surgical diagnosis. Solid nodules measuring < 8 mm are infrequently malignant, difficult to biopsy, risky to resect, and not reliably characterized by PET scan or other functional imaging tests, leaving CT scan surveillance as the most appropriate option. At this time, the frequency and duration of surveillance are guided by expert consensus-based recommendations from the Fleischner Society. Subsolid nodules often are premalignant or malignant and may require extended-duration surveillance for growth or development of a solid component. Further research is needed to weigh the benefits and harms of alternative methods for evaluating both solid and subsolid nodules. Research priorities include developing and validating risk assessment models to estimate the probability of cancer among individuals with small nodules or subsolid nodules, performing studies that compare the benefits and harms of alternative management strategies among individuals stratified by cancer risk, determining the safety of CT scan surveillance by examining outcomes among individuals who choose this strategy, and developing and validating novel noninvasive biomarkers to facilitate diagnosis and determine prognosis.

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Detection in blood of autoantibodies to tumour antigens as a case-finding method in lung cancer using the EarlyCDT®-Lung Test (ECLS): study protocol for a randomized controlled trial

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Abstract

Background: Lung cancer is the most common cause of cancer related death worldwide. The majority of cases are detected at a late stage when prognosis is poor. The EarlyCDT®-Lung Test detects autoantibodies to abnormal cell surface proteins in the earliest stages of the disease which may allow tumour detection at an earlier stage thus altering prognosis.

The primary research question is: Does using the EarlyCDT®-Lung Test to identify those at high risk of lung cancer, followed by X-ray and computed tomography (CT) scanning, reduce the incidence of patients with late-stage lung cancer (III & IV) or unclassified presentation (U) at diagnosis, compared to standard practice?

Methods: A randomised controlled trial of 12 000 participants in areas of Scotland targeting general practices serving patients in the most deprived quintile of the Scottish Index of Multiple Deprivation. Adults aged 50–75 who are at high risk of lung cancer and healthy enough to undergo potentially curative therapy (Performance Status 0–2) are eligible to participate. The intervention is the EarlyCDT®-Lung Test, followed by X-ray and CT in those with a positive result. The comparator is standard clinical practice in the UK. The primary outcome is the difference, after 24 months, between the rates of patients with stage III, IV or unclassified lung cancer at diagnosis. The secondary outcomes include: all-cause mortality; disease specific mortality; a range of morbidity outcomes; cost-effectiveness and measures examining the psychological and behavioural consequences of screening.

Participants with a positive test result but for whom the CT scan does not lead to a lung cancer diagnosis will be offered 6 monthly thoracic CTs for 24 months. An initial chest X-ray will be used to determine the speed and the need for contrast in the first screening CT. Participants who are found to have lung cancer will be followed-up to assess both time to diagnosis and stage of disease at diagnosis.

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Discussion: The study will determine the clinical and cost effectiveness of EarlyCDT®-Lung Test for early lung cancer detection and assess its suitability for a large-scale, accredited screening service. The study will also assess the potential psychological and behavioural harms arising from false positive or false negative results, as well as the potential benefits to patients of true negative EarlyCDT lung test results. A cost-effectiveness model of lung cancer screening based on the results of the EarlyCDT Lung Test study will be developed.

Trial registration: NCT01925625. August 19, 2013

Keywords: Lung cancer, Early diagnosis, Screening, Health economics, RCT, Primary care, Biomarker, Autoantibodies

Background

Lung cancer is the world's leading cause of cancer related mortality and a major source of morbidity [1]. It is often diagnosed at an advanced stage with 85% of patients undiagnosed until the disease is symptomatic [2]. Scotland has one of the highest rates of lung cancer in the world [3]. Around 2 460 men and 2 340 women are diagnosed with lung cancer in Scotland every year, which is 16% of the total UK cases, despite Scotland having only 8% of the UK's population. Survival from lung cancer is poor with less than 9% of patients still alive at 5 years after diagnosis, due primarily to the late stage of presentation [4]. Early detection and diagnosis of cancer improves prognosis - the current 5-years survival rate is approximately 60% for stage I lung cancer but is only 1% for those with stage IV disease [5].

The first studies evaluating screening for lung cancer utilised chest X-ray and/or sputum cytology [6–9]. While these showed increased numbers of earlier-stage, resectable cancers and improved survival rates in the screened groups, not all studies were randomised. The lack of trial strength data means that differences in lung cancer mortality between those screened and those not are difficult to interpret.

The National Cancer Institute National Lung Screening Trial (NLST) reported that CT screening reduced lung cancer mortality by 20% [10]. This has led to a number of guidelines in the United States which advocate lung cancer screening with low dose CT [11]. However as a primary screening modality CT is expensive and leads to a significant percentage of false positives (>90% of nodules are found to be benign) [12]. There was a substantial increase in morbidity associated with further investigation. More recently the UK Lung Cancer Screening Trial reported successful early detection of lung cancer using low dose CT scans [13].

The EarlyCDT®-Lung Test is a novel Autoantibody(-AAB) diagnostic test for the early detection of lung cancer allowing stratification of individuals according to their risk of developing lung cancer [14]. This could permit a targeted approach to CT scanning for early lung cancer detection which may be a more cost-effective and potentially less harmful approach to population screening.

The EarlyCDT®-Lung Test measures seven AABs; p53, NY-ESO-1, CAGE, GBU4-5, HuD, MAGE A4 & SOX2. It identifies 41% of lung cancers with a high specificity of 90% [14]. This compares to CT scanning, which when used alone as a prevalence screening test, identifies 67% of lung cancers developing over the following 12 months, but has a low specificity of around 49% [10]. The autoantibodies detected in the test have not been shown to vary with age, gender and ethnicity [15].

In a large group of patients ($n = 3\ 376$) with newly diagnosed lung cancers there was no difference in positivity rate for the test in early or late stage disease lung cancers, and this applied to all lung cancers [14, 16]. Thus, while autoantibodies are present in early stage they are not simply a biomarker of early stage disease. While preliminary data shows promise there is insufficient evidence, as yet, to support the introduction of this test for cancer screening or a case finding program.

Consequently, the primary research question is: 'Does using the EarlyCDT®-Lung Test, followed by X-ray and CT scanning, to identify those at high risk of lung cancer reduce the incidence of patients with late-stage lung cancer (III & IV) or unclassified presentation (U) at diagnosis, compared to standard clinical practice?

AIMS

To assess the effectiveness of the test in increasing early stage lung cancer detection, thereby reducing the rate of late stage (III/IV/U) presentation compared to standard practice; to assess the cost-effectiveness of the test compared to standard practice; to assess the impact of the test on quality of life, positive and negative affect, illness perceptions, lung cancer risk perception, health anxiety, lung cancer worry, subjective stress related to screening, smoking behaviour and health service use.

Methods

Design

This is a randomised controlled trial involving 12,000 participants recruited through primary care and community based recruitment strategies in Scotland. < h3 > Setting.

General practices who serve patients in the lowest quintile of deprivation in Scotland, as measured by the Scottish Index of Multiple Deprivation, will be targeted [17]. Additional recruitment will be attained through adverts, posters, flyers and community based interactions and may extend to other practices as needed to ensure reaching our recruitment targets. Potential participants can either be seen at their participating GP practice or at the local clinical research centre, or other appropriate clinical location.

Participants

Adults aged 50–75 who have at least a 2% risk of developing lung cancer over the next 24 months will be eligible to participate [18]. These are defined as those who are, current or former cigarette smokers with at least 20 pack-years, or have a history of cigarette smoking less than 20 pack-years plus an immediate family history (mother, father, brother, sister, child) of lung cancer which gives an individual a personal risk similar to a smoking history of 20 pack years. Participants should be healthy enough to undergo radical treatment either by pulmonary resection or stereotactic radiotherapy.

Number of participants

We will recruit 12,000 participants, from approximately 170 general practices.

Inclusion criteria

1. Participant is willing and able to give informed consent for participation in the study
2. Male or female aged 50–75 years
3. Current or Ex-smoker with at least 20 years pack history
4. Less than 20 years pack history but with family history of lung cancer in a 1st degree relative (mother, father, sister, brother, child)
5. Eastern Co-operative Oncology Group Status: 0, 1 and 2 [19]

Exclusion criteria

1. History of any cancer other than non-melanomatous skin cancer and/or cervical cancer in situ.
2. Complaining of symptoms suggestive of lung cancer within past 6 months i.e. haemoptysis or weight loss.
3. Patients for whom the GP considers invitation to the study would cause undue distress.
4. Patients with terminal disease.
5. Patients on prolonged/continuous use (>3 months) of Cyclophosphamide.

Randomisation

Participants will be allocated to the intervention or comparison group during the recruitment visit (Visit 1) using a web-based randomisation system TRuST [20]. Randomisation will be stratified by site and minimised by age, sex and smoking history.

Dates and duration of trial

01/08/2013–31/07/18 (60 months).

Identifying participants

Practices in the most deprived areas will be approached by facilitators in the Scottish Primary Care Research Network (SPCRN) to participate. Potentially eligible individuals will be identified from GP medical records by an electronic medical record search [21]. Potential participants will be recruited via their General Practitioner and a range of other methods as recommended by the pre-trial focus groups [22]:

- postal invitation letter including a summary of the study Participant Information Sheet and a full Participant Information Sheet or Participant Information Brochure for those interested;
- invitation letter including a summary of the study Participant Information Sheet on collection of repeat prescription;
- invitation during consultation with GP/Practice Nurse/Health Care Assistant at the practice;
- invitation to those eligible on registered research volunteer databases
- poster present in the GP's waiting room
- media campaign involving:

◦ local and national newspaper
◦ radio
◦ celebrity endorsement
◦ publicity campaign using posters/leaflets

The study invitation letter will include a slip for participants to either express interest in finding out more about the study [23]. Those returning an expression of interest will be telephoned, more than 24 h after anticipated receipt of the Participant Information Sheet, by a member of the research team. The call will allow a discussion of the study, to answer any questions the potential participant may have, do a preliminary assessment of eligibility and if agreed, to make an appointment for a recruitment visit. An appointment letter/email will be sent out to confirm appointment. A reminder call/email or text, whichever is preferable to the participant, will be carried our 2 days prior to the screening appointment to reduce non-attendance [24]. Non-responders to the

postal invite will be contacted by letter again once or via a message on the right side of a repeat prescription [25]. Those returning an expression of interest will be sent a full information sheet and dealt with as above.

Initial consultation

The following procedures will be undertaken in the order given below:

- obtain consent
- take bloods from all consented participants
- complete study questionnaire
- randomise to treatment arm

Administration of the test

After randomisation, all participants will be asked if they still wish to take part in the trial and still agree for their bloods to be used for the test and for future cancer related research. For participants randomised to the intervention arm the EarlyCDT®-Lung test will be performed and patients followed up according to their result (see Additional file 1: study flowchart).

At the initial visit, participants are told that those with a positive EarlyCDT®-Lung Test result will be invited to a follow-up visit to discuss the test results and explain what happens next. Those with a negative EarlyCDT®-Lung Test result will receive a letter explaining the test results and will be offered a follow-up visit or a telephone call if they wish. They will be told that the best way to reduce risk of developing lung cancer is by stopping smoking and that symptoms to watch for include persistent cough, coughing up blood, shortness of breath, weight loss or loss of appetite.

Those in the control arm will be written to and thanked for their contribution to the study and advised and counselled identically to those in the intervention arm who have had a negative EarlyCDT®-Lung Test result.

A patient specific section of the study website (www.eclsstudy.org) containing Participant Information Sheets and research staff contact details will be available for participants.

Management of the visits

Based on the test's reported 90% specificity and 41% sensitivity we anticipate that 520–550 participants in the intervention arm will have a positive test result. These will be offered a chest X-ray in accordance with local requirements for prioritisation and will be referred for a non-contrast thoracic CT scan. If there is a suspicious opacity on the chest X-ray or initial CT scan a contrast enhanced staging CT will be undertaken. As a quality control measure no participant undergoing CT screening in the test positive arm will have all their 5 CTs reported by same radiologist. Nodule size will be currently reported as the mean of 2 diameters at 90° angles, volumetric analysis is starting soon on both sites with diameter and volume to be reported. If the initial CT scan reveals no evidence of lung cancer then subsequent CT scans will be offered 6 monthly for 24 months. An appointment window of ± 4 weeks will be initiated for each scheduled CT scan.

If a test positive participant has had a chest X-ray in the previous 1 month, or a CT scan in the previous 3 months, these can be reviewed as part of the study. With the participant's consent chest X-rays or CT scans prior to study entry will be retrospectively coded. The participant will proceed to have the series of up to 5 CTs.

Participants will receive appointments via post/email, according to patient preference. Participants will be called 2–4 days before each CT scan appointment. Individuals with abnormalities as classified by the radiology/respiratory physician's study panel on baseline CT scan or subsequent CT scan will be followed up over the study period or referred for NHS clinical care as appropriate. All individuals entering the study will be flagged and followed-up via the Scottish Cancer Registry in the Electronic Data Research and Innovation Service (eDRIS) [26]. Participants who develop lung cancer will be followed-up via their medical records to assess both time to diagnosis and stage of disease at diagnosis. If no histological stage is available, stage will be assessed by a panel of three respiratory physicians blind to allocation status of the study subjects from chest X-rays or CT, or if no imaging is available, medical assessment of stage will be carried out.

Prior to sending CT scan appointment dates, the Scottish Community Health Index national register will be checked for vital status. All participants in the test- Positive test groups known to have died will be removed from the CT scan appointment schedule register. If patients (positive test) fail to attend for any imaging assessment during the study, they will receive two reminders (one letter, one phone call). On the third non-attendance, a letter will be sent to the participant's GP to inform them of non-attendance.

Participants will receive results letters in relation to their initial chest X-ray and CT scan and subsequent CT scans. Any clinical intervention/treatment will be arranged by the relevant NHS multidisciplinary team.

Control

The comparator is UK standard clinical practice which involves awaiting the development of symptoms and investigation of those symptoms according to national guidelines [27, 28].

Intervention

EarlyCDT®-Lung Test blood sample followed by X-ray and serial cross sectional CT imaging in those with a positive result 6 monthly for 24 months. Those with a

negative test, like the controls, have no further investigations but are provided with standard clinical care.

Outcomes

Primary

The difference, at 24 months after randomisation, between the rates of patients with stage III, IV or unclassified lung cancer at diagnosis in the intervention arm, and those in the control arm;

Secondary

1. numbers at 24 months after randomisation, in the different stages at diagnosis (III/IV/U/other) in the intervention arm and the control arm;
2. difference, after 24 months, between costs and outcomes between the intervention arm and in the control arm and cost-effectiveness of the test compared to standard practice;
3. differences, after 24 months, of lung cancer mortality, all-cause mortality and cancer-specific mortality rates between the intervention arm and in the control arm;
4. differences, after 5 and 10 years, of long-term future mortality rates in the intervention arm and in the control arm;
5. differences, after 24 months in (i) the number of patients with stage III, IV or unclassified lung cancer at diagnosis in the test-positive group and those in the test-negative group and (ii) stage at diagnosis in the test-positive and test-negative group;
6. difference between the test-positive, test-negative groups and the control arm at 1, 3, 6, and 12 months in scores for EQ5D [29], Positive and Negative Affect Schedule [30], revised Illness Perception Questionnaire adapted to refer to lung cancer and lung cancer risk [31], Lung cancer risk perception, Health anxiety subscale of Health Orientation Scale [32], the Adapted Lung Cancer Worry Scale [33] and Impact of Events Scale [34] (for the test-positive group, the test-negative group only) and differences in smoking behaviour and health service use. Long-term scores for the same outcomes for the test-positive group at 18 and 24 months;
7. difference in incidence at 24 months, and after 5 and 10 years, in other clinical measures such as Cerebrovascular disease, Chronic Obstructive Pulmonary Disease, hospital stays, and outcomes identified through the Scottish Morbidity Record (SMR) linkage in the intervention arm and in the control arm
8. numbers in all groups at 24 months (test-positive, test-negative and control) undertaking subsequent investigations such as chest X-ray, CT and bronchoscopy (Table 1)

Statistics and data analysis

Sample size calculations

Main study The rate of lung cancer was 187/100,000 per year for patients aged 50–74 in Scotland 2008 which is higher than many other similar countries [23]. Deprivation is associated with a significantly higher risk of lung cancer. Living in the most deprived quintile is associated with an increased risk of 1.8 times compared to the middle quintile of deprivation; this gives an estimated annual lung cancer rate of 336/100,000 among the practices taking part in the study. A high risk group within this population will be selected using similar entry criteria (outlined above) as the Mayo screening study which had a 2% prevalence rate of lung cancer and a further 2% incidence rate over the following 5 years [35]. The baseline rate of late stage presentation for the particular high risk population envisaged in this study is uncertain, as is the size of the reduction in late stage presentation likely to be achieved through use of EarlyCDT®-Lung Test. Using an estimated late stage presentation rate of 1,200/100,000 per year in the control arm i.e. 2.4% over the 2-years follow-up period, provides 85% power at 5% significance (two-sided) to detect an estimated reduction of 35% in late stage presentation rate in the intervention arm i.e. as low as 780/100,000 per year or 1.56% over the 2-years follow-up period. This corresponds to an estimated event rate over the 24 months of follow-up of 120 events in the control arm and 78 events in the intervention arm and implies a required sample size of 5,000 per arm i.e. a total of 10,000 participants.

The anticipated 35% reduction in event rate between the control arm and the intervention arm was justified by current estimates of the capability of the test to identify cases together with current estimates of the sensitivity of CT scanning (67%). The assumed event rate in the study participants of 1.2% per year was an estimate and the sample size would be modified if the observed event rate proves to be markedly different, acknowledging the a priori possibility that we will employ a prospective adaptive design. No Interim analysis of efficacy is planned.

The sample size calculations are based upon standard methods for time to event data using the c power function in R and st power exponential procedure in Stata and assuming exponential survival [34, 36]. They were also confirmed using standard approaches for detecting a change in binomial probabilities, and confirmed using approaches to detect a change in Poisson rates (with essentially identical results as loss to follow up is expected to be low due to completeness of Scottish Morbidity Register data).

Table 1 Data Collection Timeline

Assessment/Procedures	Timeline (± 2 weeks)				
	Visit 1 (~30-45mins)		Visit 2 (~30mns)	➢ *EarlyCDT Positive Test Participants may visit or call.* ➢ *EARLY CDT Negative Test Participants may attend for further information/advice only.*	
Informed Consent	X				
Inclusion/Exclusion Criteria	X				
• *Review/Record only Relevant Medical History relating to IC/EC*	X				
• *Review/Record Relevant Medications* • *Relating to IC/EC*	X				
Blood Sample	X				
Baseline Questionnaire	X				
Thank you letter to Control Group		X			
EarlyCDT- Lung Test Result Letter		X			
GP Results Letter & ICF copy (negative)		X			
Result Discussion/ Imaging Schedule			X		
Provide PIS 2			X		
GP Result Letter & ICF copy (positive)			X		
EarlyCDT – Lung Test Positive Result Participants – Imaging Schedule					
	Timeline(± 12 weeks)				
	0	6 months	12 months	18 months	24 months
CXR	X				
CT Scan	X	X	X	X	X
Scheduled every 6 months, if participant enters NHS clinical care pathway, subsequent study CT scans will be cancelled.		*Research team member will call 2–4 days before each scheduled CT scan to check health status and attendance.*			

The study aims for a short recruitment period and so no allowance has been made for accrual. With such an allowance, say to 1 year, the power will increase to 91% to identify a 35% reduction provided the minimum follow up period of 2 years is observed.

The initial assumptions of the rate of late stage presentation rate of 1,200/100,000 per year among the study participants was too optimistic and in January to May 2015 investigations were carried out to inform an increase in the sample size. Baseline information on the 8639 participants recruited to March 2015 (18 months from first randomisation) was used to derive an estimate of lung cancer risk based upon the Spitz Model. A number of variables in this model were not recorded in the study data base and low risk values were used in the risk calculation implying that the risk estimates should be underestimates. This suggested that the with 10,000 participants the rate of lung cancer would be expected to be around 680/100,000 and 540/100,000 for stage T3/T4/Unknown lung cancer using ISD cancer statistics figures of 80% lung cancers in Scotland are late stage. A sensitivity analysis around the missing data assumptions suggests that a late stage rate of around 600/100,000 may not be unreasonable, though is likely to be at the upper limit.

Using an assumption of 600/100,000 for late stage lung cancer, increasing the sample size to 12,000 [37], and acknowledging that recruitment is over a 2 years period the study has a power of 80% to detect a 35% reduction associated with the use of the EarlyCDT-Lung test to identify cases, provided that analysis takes place after all randomised patients have been followed up for 2 years. While an 80% power is at the lower end of acceptable powers this is the power level which has been used in a number of lung cancer screening trials.

The power of the study is sensitive to the assumptions about the rate of late stage cancer and the recruitment rate. A power in excess of 90% could only realistically be achieved by recruiting 15,000 patients or by changing the primary endpoint to 3 years post randomisation for all patients. If the recruitment phase extends past 2–2.5 years to recruit 12,000 participants then the power will increase slightly to 83%.

Substudies For the follow-up analysis of behavioral and psychological outcomes, 200 participants in each group (test-positive, test-negative and the control arm) will allow detection of a mean difference of 3.00 (SD 15.04

(unpublished data comparing pre and post prostate biopsy scores from the ProtecT prostate cancer study)) in the Impact of Events Scale between baseline and follow up measurements. (http://www.ncbi.nlm.nih.gov/pubmed/21047592) This study reported within each group and a mean difference of 4.2 (SD 15.04), between each of the test groups and the control arm with 80% power and 2-sided 5% significance level. Assuming 80% of participants are current smokers, this will provide 80% power at 5% significance level to detect a 13% point difference in the prevalence of smoking between each of the test groups and the control arm (i.e. from 80 to 67%) To allow for attrition, we will recruit 300 participants in each group.

Proposed analyses

Characteristics of participants will be compared informally between treatment arms at baseline. The main analysis of the primary outcome will be intention-to-treat. Cox proportional hazards models which will be used to estimate the hazard ratio of the rate of late stage lung cancer in the intervention arm compared to the control arm. Participants who are lost to follow up will be censored. The models will adjust for age, gender smoking history, socioeconomic status and practice. If appropriate, random cluster effects will be included rather than fixed effects for practices. A similar methodology will be used for the secondary outcomes of comparisons of mortality rates. A subsequent analysis will compare the outcomes of those with a positive test in comparison to those in the intervention group with a negative test (primary contrast for this analysis) and those in the control group. Comparisons of proportions will be carried out using chi square tests. Fisher's exact test will be used if the number of events is small.

Psychological and behavioral outcomes will be compared between the three groups (Test-positive, Test-negative and the control group) at baseline using analysis of variance (or non-parametric tests if there is evidence of non-normal distribution of scores) for continuous measures and χ^2 tests for categorical measures. Psychological (HADS Score) and behavioral measures will be described at each follow up time point and multilevel regression models will be used for analyses to take account of repeated measurements during follow up [38].

Poisson regression models, adjusting for follow up time if necessary, will be used to investigate the other clinical measures (secondary outcomes 7 and 8).

Cost effectiveness analysis

A short-term within-trial analysis will compare the costs and outcomes associated with the intervention arm to those of the comparison arm at 24 months, with a focus on cost-per-case detected. A longer term analysis will employ a decision analytic model to link the short term outcomes measured within the trial to potential longer term impacts on health, in terms of impacts on morbidity and mortality of early detection and treatment, to allow the estimation of cost-per Quality Adjusted Life Years gained. Both analyses will take the perspective of the NHS and personal social services and conform to the reference case favoured by NICE [39].

Missing data

The extent of missing data will be examined and, if necessary, methods such as multiple imputation will be implemented to assess the robustness of results to missing data, assuming data are missing at random.

End of study

The end of study is defined as last patient last visit test-related scan plus 24 months. The Sponsor, CI and/or the Trial Steering Committee have the right at any time to terminate the study for clinical or administrative reasons.

Data collection & management

Data collection

All research blood samples will be transported to the University of Nottingham for processing, and then transported to the US for Test processing by Oncimmune. All samples will be stored under custodianship as per UK Biobank guidelines [40]. Sample Analysis and Chain of Custody Plans are documented in the Study Operations Manual. The participant's medical notes (GP and hospital) paper or electronic will act as source data for relevant past medical history, subsequent medical conditions, hospital admissions and diagnostic reports.

Psychological and behavioural data will be collected on the first 10,000 participants through a baseline questionnaire administered during Visit 1. Follow-up data will be collected between 1 and 12 months on subsets of the intervention and control arms and at 18 and 24 months for the EarlyCDT®-Lung Test-positive group. Data collected at baseline will include the EQ5D, Hospital Anxiety and Depression Scale, Positive and Negative Affect Schedule, revised Illness Perception Questionnaire adapted to refer to lung cancer and lung cancer risk, lung cancer risk perception, items from the Health Orientation Scale, the adapted Lung Cancer Worry Scale, smoking behaviour and demographic details. Follow-up questionnaires include the same measures, plus health service use and Impact of Events Scale and health service use for those who had the test. The Hospital Anxiety and Depression Scale is not included in follow-up questionnaires and the EQ-5D is not included in the 3 months follow-up questionnaire.

All participants in the positive group will be approached with the recruitment aim of 300 from this group. The TCTU will use an electronic randomisation tool to randomly sample patients from the test-negative

group and control arm, stratified by the two study centres. Twenty-one individuals will be sampled each week and invited to complete follow-up questionnaires with an aim of recruiting 300 from both groups (based on an anticipated response rate of 67%).

Participants who receive a diagnosis of lung cancer will not be followed up subsequent to receiving the diagnosis.

Data management and data management system

Data will be collected by the RN either directly onto a paper CRF with subsequent transcription to the eCRF, or direct data entry onto the web based eCRF.

TCTU will provide a data management system using OpenClinica [41]. The data management system will be fully validated, including the provision of test data and supporting documentation. Backup and disaster recovery will be provided by TCTU according to its standard operating procedures [42].

The Statistical Analysis Plan will specify dummy tables linked to primary and secondary outcomes and the data management system will be designed to export directly to the dummy table formats for analysis.

Safety assessments

Adverse Events (AE) and Serious Adverse Events (SAE) will be recorded. A number of factors affecting the trial population suggest that we would expect to observe a larger than normal incidence of episodes of ill-health due to both the age and co-morbidities of the study population. All chest X-ray and CT scan incidental findings will be recorded in the CRF as an incidental finding and a specialist referral will be made as directed in a study Standard Operating Procedure (SOP) within the Study Operations Manual [43]. AEs (as defined) will be recorded as soon as they are known either from the study subjects, PI patient review audits or via SMR or record review.

Ethical considerations

The study will be conducted in accordance with the principles of good clinical practice (GCP) and the Research Governance Framework Scotland [44].

Confidentiality

All records will be kept in a secure storage area with limited access to study staff only. Clinical information will not be released without the written permission of the participant, except as necessary for monitoring and auditing by the Sponsor, its designee or Regulatory Authorities.

Data protection

The CI and study staff involved with this study will comply with the requirements of the Data Protection Act 1998 with regard to the collection, storage, processing and disclosure of personal information and will uphold the Act's core principles. The CI and study staff will also adhere to the current version of the NHS Scotland Code of Practice on Protecting Patient Confidentiality and all other governance requirements. Published results will not contain any personal data that could allow identification of individual participants.

Insurance and indemnity

The University of Dundee and Tayside Health Board are Co-Sponsoring the study.

Discussion

Despite advances in surgical techniques, radiation therapy and systemic therapy the outlook for patients with lung cancer has improved more slowly than many other cancers over the last 50 years. Early diagnosis of treatable disease is likely to be the major way of changing outcomes for the foreseeable future. The study will assess the EarlyCDT®-Lung Test's clinical suitability and cost effectiveness for a large-scale, accredited screening service for early lung cancer detection. It will also assess potential morbidity arising from the test and potential psychological and behavioural harms and benefits of test results.

The major strength of this trial lies within its design. By being both randomised and controlled many of the inherent biases that affected many of the previous screening studies will be removed. Our trial will also be able to investigate the effect of either a positive or negative result on participants' lifestyle decisions to explore whether a negative result reinforces harmful behaviour, such as smoking, or a positive result reduces harmful behaviour. To date, screening with low dose CT scanning does not appear to have a beneficial effect on smoking behaviour [38, 45–47]. As in many screening studies a potential weakness is that the study population may be different to the usual clinical populations in terms of age, smoking status and education. Our eligibility criteria ensure participants have a high risk of lung cancer over the subsequent 24 months and our findings should be generalisable to populations with a similar level of risk. Our focus on areas with high levels of deprivation for recruitment should help ensure our participants reflect the social gradient in lung cancer incidence and our collection of demographic data will enable us to compare the characteristics of our trial population with those at risk of lung cancer.

One potential weakness is that participants randomised to the control arm may change their behaviour to decrease their risk of lung cancer e.g. by stopping smoking in a way that they would not have done had they not been participating in our study. The lack of impact of

screening using CT scanning on smoking behaviour suggests this may not occur to an important extent, but measurement of smoking behaviour at repeated follow up time points will enable us to quantify this and assess its impact on our findings.

Trial status

Recruitment began the 7th of August 2013.

Additional file

Additional file 1: Figure schedule of enrolment, interventions, and assessments. (DOC 53 kb)

Abbreviations

AAB: Autoantibody; AE: Adverse event; CI: Chief investigator; CNORIS: Clinical negligence and other risks scheme; CRF: Care report form; CT scan: Computerised tomography scan; EarlyCDT®-Lung Test: Early cancer detection test; ECLS: Study early cancer detection test - lung cancer Scotland study; eCRF: Electronic case report form; eDRIS: Electronic data research and innovation service; GCP: Good clinical practice; HIC: Health informatics centre; IATA: International Air Transport Association; ICF: Informed consent form; ISF: Investigator site file; NLST: National lung screening trial; PANAS: Positive and negative affect schedule; RN: Research nurse; SAE: Serious adverse event; SCR: Scottish cancer register; SMR: Scottish morbidity record; SOP: Standard operating procedure; TAA: Tumour derived/associated antigens; TASC: Tayside medical science centre; TCTU: Tayside clinical trials unit; TMF: Trial master file

Funding

Funding for the study was provided by the Chief Scientist Office, Scottish Government and Oncimmune Ltd.

Availability of data and materials

The manuscript dos not rely upon any datasets but it is intended that the data and samples produced during the study will be deposited in publicly available repositories.

Authors' contributions

FS conceived of the study, and participated in its design and coordination and helped to draft the manuscript. EF helped to draft the manuscript. FM participated in the coordination of the study and helped to draft the manuscript. ST participated in the coordination and participated in its design of the study and helped to draft the manuscript. SJ participated in the coordination of the study and helped to draft the manuscript. CJ participated in the coordination of the study and helped to draft the manuscript. CR participated in the coordination and participated in its design of the study and helped to draft the manuscript. AB participated in the coordination of the study and helped to draft the manuscript. CMcC conceived of the study, and participated in its design and coordination and helped to draft the manuscript. ST conceived of the study, and participated in its design and coordination and helped to draft the manuscript. DK conceived of the study, and participated in its design and coordination and helped to draft the manuscript. KV conceived of the study, and participated in its design and coordination and helped to draft the manuscript. LB participated in the coordination of the study and helped to draft the manuscript. BY participated in the coordination of the study and helped to draft the manuscript. SG participated in the design and coordination of the study and helped to draft the manuscript. RL is the senior trial manager, participated in its design and coordination and helped to draft the manuscript. JR conceived of the study, and participated in its design and coordination and helped to draft the manuscript. HS conceived of the study, and participated in its design and coordination and helped to draft the manuscript. AD conceived of the study, and participated in its design and coordination and helped to draft the manuscript. SS participated in the study design and coordination and drafted the manuscript. All authors read and approved the final manuscript.

Competing interests

The ECLS study is part funded by Scotland's Chief scientist office and Oncimmune http://www.oncimmune.co.uk; the funders have no involvement in the conduct of the study, analysis, data interpretation or publication of results.
Herb Sewell: current ECLS study is part funded by Oncimmune; has share options with Oncimmune.
Chris Robertson: Paid Consulting work for Oncimmune on the development of their test. This was from 2009–12. Signed a non-disclosure agreement. Stock Options with Oncimmune.
John Robertson: Shares & share options in Oncimmune. Otherwise hasn't been involved in the company for over two years.

Consent for publication

No individual person's data in any form (including individual details, images or videos) are included.

Ethics approval and consent to participate

Ethical approval was given by the Tayside research ethics committee, reference NRS13/ON400.

Consent

Informed consent will be obtained from each study participant. All individuals taking informed consent will have received training in Good Clinical Practice (GCP). It will be explained to patients that they are under no obligation to enter the trial and that they can withdraw at any time during the trial, without having to give a reason.

Publisher's Note

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Data at November 2016	
Participants randomised	**12 210**
Participants in No Test Group	6 120
Participants in Test Group	6 091
Negative Early CDT Lung test results	5 442
Positive Early CDT Lung test results	595
Invalid Early CDT Lung test results	4
% Positive Early CDT Lung test results	9.8%
Chest X-rays completed	590
CT scans completed	2 080
Nodules	277
Participants undergoing further investigations	1
Lung cancers diagnosed	**16**
Early Stage Cancers	**12**

SCHOOL OF MEDICINE

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Data at end of July 2017	
Participants randomised	**12 210**
Participants in No Test Group	6 121
Participants in Test Group	6 089
Negative Early CDT Lung test results	5 486
Positive Early CDT Lung test results	598
Invalid Early CDT Lung test results	2
% Positive Early CDT Lung test results	9.8%
Chest X-rays completed	583
CT scans completed	2 355
Nodules	327
Participants undergoing further Investigations (and staging)	10(1)
Lung cancers diagnosed	**16**
Early Stage Cancers	**12**

SCHOOL OF MEDICINE
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*Early*CDT-Lung

Regulatory Information

STAGE ZERO LIFE SCIENCES

CE MARK APPROVAL

On July 15, 2016 Oncimmune announced that it had received a European CE for its product *Early***CDT-Lung**. The CE mark certifies that the reagents used in the *Early***CDT-Lung** test meet the European Union's standards for quality control and manufacturing, to enable the product to be placed on the market.

NYSDOH APPROVAL

On September 20, 2011 Oncimmune announced that *Early***CDT-Lung** was approved by the New York State Department of Health. Oncimmune was awarded a Clinical Laboratory Permit by the New York State Department of Health on August 31, 2011. The process included a comprehensive review of clinical laboratory operations, materials, validation methods and data, as well as a thorough onsite inspection of the laboratory.

REGULATORY INFORMATION

Oncimmune's testing is performed in a registered, high-complexity laboratory. They meet or exceed all CLIA and CAP regulations and are compliant with OSHA regulations (OSHA – Occupational Safety & Health Administration).Following an assessment by BSI, Oncimmune's Quality Management System is now certified to EN ISO 13485:2012 standard for the design, development and manufacture of in vitro diagnostic immune biomarker devices for early detection of solid tumor cancers (ISO Certificate).
Oncimmune® (UK) Ltd complies with the Data Protection Act 1998.

*Early*CDT-Lung

Evaluation & Customer Support

STAGE ZERO LIFE SCIENCES

Lung Cancer Clinical Advisory Group

- Professor Peter Boyle
- Professor Herb Fritsche
- Dr. Neal Navani
- Professor Jim Jett MD: Pulmonologist, National Jewish, Denver
- Professor Frank Detterbeck: Head of Thoracic Surgery, Yale University, CT
- Professor Pierre Massion: Pulmonologist Vanderbilt, Nashville; 29 Professors
- Tim Kennedy: Pulmonologist University of Colorado
- Professor Peter Mazzone: Pulmonologist Cleveland Clinic

Evaluation & Customer Support

- More than 120,000 patient samples were run and 12 million data points analyzed to validate the technical and clinical performance of *Early***CDT-Lung**.

- More than 150,000 commercial tests have also been run in the US laboratory.

- Currently, *Early***CDT-Lung** is being used in the largest randomized trial for the early detection of lung cancer using biomarkers ever conducted; the National Health Service (NHS) Scotland ECLS study of 12,000 high-risk smokers.

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